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RESEARCH ARTICLE

Hepatoprotective Efficacy of Phytochemically Screened *Nyctanthes arbor-tristis*

Ajay Sharma[1], Shailesh M. Kewatkar[2*], Jeevan S. Dhumal[3], Gaurav Jain[4], Akanksha Jagwani[5], Amit Joshi[6] and Har Goving Garg[6]

[1]Mahakal Institute of Pharmaceutical Studies, Ujjain – 456664, Madhya Pradesh, India
[2]Rajarshi Shahu College of Pharmacy, Buldana – 443001, Maharashtra, India; kewatkar.shailesh@gmail.com
[3]Rajmata Jijau Shikshan Prasarak Mandal's College of Pharmacy, Pune – 412105, Maharashtra, India
[4]IES Institute of Pharmacy, IES University, Bhopal – 462044, Madhya Pradesh, India
[5]LNCT School of Pharmacy, LNCT Vidhyapeeth University, Indore – 452016, Madhya Pradesh, India
[6]Lakshmi Narain College of Pharmacy, Bhopal – 462022, Madhya Pradesh, India

Abstract

The *Oleaceae* plant *Nyctanthes arbor-tristis* Linnaeus is used in *Ayurveda* medicine as a laxative, diuretic, and for the treatment of a variety of painful conditions including sciatica, arthritis, and fever. This study aimed to determine whether or not extracts of *Nyctanthes arbor-tristis* leaves, either in alcohol or water, might prevent liver damage caused by carbon tetrachloride in rats. Administration of alcoholic and aqueous extracts of the leaves of *Nyctanthes arbor-tristis* protects the liver against the toxicity of carbon tetrachloride by lowering the high levels of serum glutamate pyruvate transaminase, serum glutamate oxaloacetate transaminase, and serum bilirubin (total and direct). Both the alcoholic and aqueous extracts demonstrated hepatoprotective efficacy at a dosage of 500 mg/kg body weight by significantly reducing the high levels of biochemical markers. Liver histopathology studies corroborated the extracts' potential to restore damaged hepatocytes.

Keywords: Hepatoprotective Efficacy, *Nyctanthes arbor-tristis*, Phytochemical Screening

1. Introduction

The liver is responsible for a large portion of metabolic regulation. Serum protein synthesis, digestion of dietary amino acids, carbohydrates, lipids, and vitamins, and elimination of end-of-life products and xenobiotic contaminants through bile are all part of this process. Synthetic medications are often used to cure problems that the human body cannot. India has 33 plant combinations that are covered by patents, and together they include more than 87 distinct plant species (Figure 1). India is home to a wide variety of medicinal plants. *Flacourtia indica, Annona squamosa, Silybum marianum, Cichorium intybus*, and *Aegle marmelos* are only a few of the species that have been utilised medicinally (as hepatoprotectants). The *Sida veronicaefolia* plant is the subject of several published works[1-4].

The *Nyctanthes arbor-tristis* tree, a member of the Oleaceae (*nyctaginaceae)* family, is well-known in India and its neighboring countries. A "night-blooming, sorrowful tree" is one name for this plant. The lifetime of this plant is between 5–20 years. It's a gigantic shrub that may grow to be 10 meters tall. It features simple, opposite leaflets that are between 6 and 12 cm in length and 2 and 6.5 cm in width, as well as square branches with flaky, rough grey bark. There are five to eight lobes on the corolla, and the flower's center is a vibrant orange. *Nyctanthes arbor-tristis* is happiest as a ground cover plant in dry, deciduous woodlands on rocky or sandy slopes. Its natural habitats in India include the eastern Himalayas, Assam, Bengal, the country's center area, and the southern Godavari River region. In addition, regular garden conditions are ideal for it. *Nyctanthes arbor-tristis* L. has properties such

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Figure 1. *Nyctanthes arbor-tristis*.

as antipyretic, antispasmodic, anti-inflammatory, and central nervous system depressant in both its bloom and its seed. Anti-allergic, anti-malarial, Amoebicidal, and antihelminthic properties have also been observed in this plant. This study attempts to ascertain whether *Nyctanthes arbor-tristis* stem bark hydro-ethanol extracts can safeguard the liver[5-7].

Figure 2. Tree of *Nyctanthes arbor-tristis*.

2. Materials and Methods

2.1 Collection and Authentication of the Stem Bark

Dr. S. K. Billore, professor and head of the Department of Plant and Environmental Management at Vikram University in Ujjain, Madhya Pradesh, identified and certified the stem bark of the *Nyctanthes arbor-tristis* plant that was taken from the area of the hamlet Ingoriya (Figure 2). The voucher specimen was sent to the Department of Pharmacognosy at the Mahakal Institute of Pharmaceutical Sciences in Ujjain, Madhya Pradesh. (MIPS/N/012/2010) and (MIPS/S/005).

2.2 Preparation of Extracts of *Nyctanthes arbor-tristis*

Using the stem bark of the *Nyctanthes arbor-tristis* plant, the extract was made. The leaves and stem bark of the plant were collected, let to dry in the shade, and then mechanically ground into a coarse powder. The powder was put in an airtight container after passing through a No. 40 mesh screen.

2.3 Extraction of Dried Leaves by using Various Solvents of Increasing Polarity

As part of the extraction process, *Nyctanthes arbor-tristis* stem bark was collected, sorted, cleaned, and crushed. The Soxhlet equipment accommodated 500 gm of the powdered substance. It was extracted using polar and nonpolar solvents such as petroleum ether, chloroform, acetone, and ethanol. Before they were used, the solvents were sterilized. During the 72-hr extraction method, many solvents and continuous hot percolation were utilised. The water was removed by performing maceration at a low temperature. The extracts were concentrated tenfold using vacuum distillation, and the solvent was then evaporated using a water bath and a 100 ml beaker. They were then placed in a desiccator to remove their moisture and chill them down. In jars, the dried extracts were kept and then examined[8].

2.4 Physico Chemical Evaluation

Using vacuum distillation, the extracts were concentrated. Preliminary phytochemical screening. To identify the many active constituents, such as carbohydrates, glycosides, alkaloids, amino acids, flavanoids, fixed oils, tannins, gum and mucilage,

phytosterols, etc., qualitative tests were performed on *Nyctanthes arbor-tristis* extracts. Phytoconstituents were identified using chemical analysis, and the findings indicated that unique components were present in each extract[9].

2.5 Pharmacological Studies

2.5.1 Animals

The Central Drug Research Organization in Lucknow offered for sale female Wistar albino rats weighing between 150 and 200 g for the purpose of acute toxicity testing. They were given typical rat pellets and kept in polypropylene cages (both provided by Hindustan Lever Ltd, Bangalore). The light and dark cycles were repeated every 12 hr for the rats. The use of rats in the experiment, when they were fasting (for a minimum of 12 hrs), was approved by the institution's animal ethics committee. All tests were carried out in the morning in accordance with the ethical standards set out by the CPCSEA for the study of experimental pain in conscious animals as well as the guidelines for the care of laboratory animals. Rats were given medications orally using a standard orogastric cannula.

2.5.2 Chemicals and Reagents

Inducing Agent: Paracetamol

Standard Drug: Silymarin

Test Compound: 50% hydro-ethanol extract of stem bark from *N. arbor-tristis*

2.5.3 Acute Toxicity Studies

Organization for Economic Cooperation and Development (OECD) establishes the criteria for what should be included in an oral acute toxicity investigation. For assessing a substance's toxicity when ingested, the acute toxic category technique (Guideline 423) is employed. The next step is to determine the minimum effective dosage. Two healthy young adult rats receive an oral bolus dosage of the test medication, and their behaviour is monitored for up to 15 days. All of the animals were examined daily, with attention paid to their skin, fur, eyes, mucus membranes (including the nose), respiratory rate, circulatory signs (such as heart rate), autonomic effects (such as salivation, lacrimation, perspiration, urinary incontinence, and defecation), and central nervous system (such as drowsiness, gait, tremors, and convulsions). Doses of 5, 50, 300, 2000, and 5000 mg/kg of body weight were used for the tests. This has been shown to be a lethal dose. All of the animals in this research were female, and there were three of them in each of the treatment groups[10-12].

2.5.4 Hepatoprotective Studies

2.5.4.1 Carbon Tetrachloride (CCl_4) Induced Hepatotoxicity

The nascent oxygen O^- from lipoperoxidation, which is produced when the medication is metabolized in the endoplasmic reticulum and mitochondria, leads to an increase in intracellular reactive Fe^{+2} ions, aldehyde depletion, and calcium sequestration. Ca^{+2} sequestration breakdown is facilitated by both direct covalent interaction and oxidative CCl_3 O^-. Increased intercellular Ca^{+2}, aggregation by proteolytic enzymes, and an increase in Fe^{+2} ions precipitate aldehyde cytotoxicity through lipid peroxidation in the absence of sequestration.

2.5.4.1.1 Experimental Design

- Nine groups of six rats each were created using rats of any sex. (n = 6).
- **Group I:** Received as the usual control group and received water (5 ml/kg, p.o.) once daily for 9 days.
- **Group II:** Received carbon tetrachloride (1 ml/kg in 50% v/v olive oil, S.C.) on the seventh day and water (5 ml/kg, p.o.) for the next nine days.
- **Group III:** Received carbon tetrachloride (1 ml/kg in 50% v/v olive oil, SC) on the seventh day in addition to the normal medication silymarin (25 mg/kg, p.o.) for 9 days.
- **Groups IV, V, VI, VII, VIII, and IX:** Received carbon tetrachloride (1 ml/kg in 50% v/v olive oil, SC) on the seventh day in addition to receiving all extract (500 mg/kg) once day for nine days.

On the final day, previously described techniques were used to analyte serum marker enzyme parameters, including Serum Glutamic Pyruvate Transaminase (SGPT) and Serum Glutamic Oxaloacetic Transaminase (SGOT), as well as functional parameters, including sleep onset and duration, morphological parameters including liver weight and volume, and biochemical parameters including Alkaline Phosphatase (ALP), total bilirubin, and total protein[10,13,14].

2.5.4.2 Paracetamol Induced Hepatotoxicity

The molecule responsible for paracetamol-induced liver damage is called a hepatotoxic metabolite. Sulfate and glucuronide conjugates are the main metabolites of paracetamol at therapeutic concentrations. Reactive intermediates are formed when waste compounds are combined with glutathione. More of the drug is transformed into the reactive metabolite when the sulphate and glucuronide pathways are exhausted, as occurs after an overdose. The reactive metabolite may be removed by the conjugation route if the liver is supplied with molecules that function similarly to glutathione, such as acetyl cysteine. This shields liver cells from harm and stops it from happening in the first place.

2.5.4.2.1 Experimental Design

- Nine groups of six rats each were created using rats of any sex. (n = 6).
- **Group I:** Received as the usual control group and received water (5 ml/kg, p.o.) once daily for 9 days.
- **Group II:** On the seventh day, they were given paracetamol (1 g/kg, p.o.) and water (5 ml/kg, p.o.) for the next nine days.
- **Group III:** On the seventh day, paracetamol (1 g/kg, p.o.) was administered in addition to the normal medication silymarin (25 mg/kg, p.o.) for 9 days.
- **Groups IV, V, VI, VII, VIII, and IX:** Received paracetamol (1 g/kg, p.o.) on the seventh day in addition to all extract (500 mg/kg) for 9 days.

Biochemical measures, including total bilirubin and total protein, and enzyme markers, such SGPT and SGOT, were measured on the last day of the treatment[15-18].

2.5.4.3 Ethanol Induced Hepatotoxicity

Excessive alcohol use may harm the liver in a number of ways. Hepatitis, cirrhosis, and fat buildup in the liver are the most prevalent results. In addition to any dietary deficiencies, the liver will suffer from alcohol's negative effects. Alcohol, even at low concentrations, may speed up the process by which a healthy person's liver stores fat. This accumulates when the tricarboxylic acid cycle rate and fat oxidation slow. Adding to the present situation is the fact that aldehyde and alcohol dehydrogenases produce large amounts of NADH (reduced form of nicotinamide adenine dinucleotide). Alcoholic cirrhosis causes fibrosis due to tissue death and persistent inflammation. The liver has been completely regenerated with fibrous tissue. Alcohol may have direct effects on the stellate cells of the liver, leading to an excess of collagen near the venal outflow, according to some evidence. Stellate cells become collagen-producing myofibroblasts in response to heavy alcohol intake. Mallory bodies are characteristic of alcoholic cirrhosis and have been linked to disruptions in the intermediate cytoskeleton composed of cytokeratin. There are several hypotheses on molecular processes.

2.5.4.3.1 Experimental Design

- Nine groups of six rats each were created using rats of any sex (n = 6) in each group.
- **Group I:** Received as the standard control group and received water (5 ml/kg, p.o.) once a day for 21 days.
- **Group II:** was given water (5 ml/kg, p.o.) and 40% ethanol (2 ml/l00 g b.w., p.o.) daily for 21 days.
- **Group III:** The conventional medication silymarin (25 mg/kg, p.o.) and 40% ethanol (v/v, 2 ml/l00 g b.w., p.o.) were given to Group III for 21 days each.
- **Groups IV, V, VI, VII, VIII, and IX:** received 40% ethanol (v/v, 2 ml/l00 g b.w., p.o.) for 21 days along with all extracts (500 mg/kg) every day**.**

On the last day, the stated methods were employed to analyte total bilirubin and total protein levels in addition to the other functional and morphological criteria and serum marker enzyme parameters[15,19-22].

2.5.4.4 Rifampicin Induced Hepatotoxicity

Rifampicin is the medicine of choice for treating tuberculosis, although it has been associated with liver damage when used for long periods of time. Since it competes with bilirubin for absorption by liver cells, rifampicin may be harmful to the liver. Patients with rifampicin-induced chronic hepatitis often have hyperbilirubinemia, either conjugated or unconjugated.

2.5.4.4.1 Experimental Design

- The rats were divided into nine groups of six animals in each.
- **Group I:** acted as the standard control group and received vehicle water (5ml/kg/p.o.) once daily for 21 days.

- **Group II:** During 21 days, Group II received vehicle water (5ml/kg/p.o.) and RIF+INH (100mg/kg/i.p.) on a daily basis.
- **Group III:** received silymarin (25 mg/kg/p.o.) for 21 days and RIF+INH (100 mg/kg/i.p.) for 21 days.
- **Groups IV, V, VI, VII, VIII, and IX:** were given RIF+INH (100mg/kg/i.p.) for 21 days once a day and all of the chosen plants extract (500mg/kg/p.o.) for 21 days once a day.

On the last day, the stated methods were employed to analyte total bilirubin and total protein levels in addition to the other functional and morphological criteria and serum marker enzyme parameters[23-25].

2.6 Histopathological Studies

The livers were removed at slaughter, cut thinly, and preserved in 10% formalin for two days to prevent spoilage. The liver sample was dehydrated three times for 12 hrs each using 70% to 90% isopropyl alcohol after being washed under running water for 12 hrs to remove the formalin. When a person consumes nothing but alcohol for three to five consecutive 12-hr intervals, they begin to dehydrate. Any remaining moisture was removed by dehydration. In order to remove the ethanol, first chloroform was employed, and then paraffin penetration was performed. Two 1520 min sessions of chloroform cleaning were performed. Paraffin-coated liver slices were then processed by a tissue-processing robot. L-shaped blocks with vacuum-embedded holes were created by pouring molten, solid paraffin. Paraffin wax was heated, and tiny chunks of liver were added. The blocks were sectioned off using a microtome into 5-m thick pieces. The slices were placed on an egg albumin-coated microscope slide[24-27].

3. Results and Discussion

3.1 Physicochemical Analysis of Crude Drug

Research on the hepatoprotective characteristics of *Nyctanthes arbor-tristis* plant stem bark was chosen based on a study of the relevant literature and interviews with traditional medicinal practitioners from Ujjain, Bhanpura, and Bhopal in Madhya Pradesh, India. A physicochemical examination was performed on the powdered leaves and stem bark. Ashes were measured in this study in three different ways: total ash, acid-insoluble ash, and water-soluble ash. *N. arbor-tristis* has a high concentration of inorganic materials, as shown by the total ash content of its stem bark, which was determined to be 4.55% w/w. It was discovered that *N. arbor-tristis* had 2.25 per cent of acid-insoluble ash in its stem bark. *N. arbor-tristis* has been shown to have 2.7% w/w water-soluble ash in its stem bark. Table 1 displays the findings.

Table 1. Ash values of stem bark of *N. arbor-tristis*

Plant Name	Part Used	Types of Ash	Percentage of Ash(w/w)
N. arbor-tristis	Stem bark	Total ash	4.55
		Acid Insoluble	2.25
		Water soluble	2.7

3.2 Determination of Extractive Value

Extractive values determined were showed in Table 2. The extractive values indicate the presence of considerable amount of phytoconstituents in solvents.

Table 2. Extractive values of stem bark of *N. arbor-tristis*

Solvent	% Yield
	N. arbor-tristis
Pet. Ether	1.89
Chloroform	1.54
Acetone	15.8
Ethanol	10.5
Aqueous	19.2

3.3 Preliminary Phytochemical Analysis

Chemical studies showed that different extracts included identifiable components, which allowed for their isolation and identification. The results showed that acetone, ethanol, and water extracts of *N. arbor-tristis* stem bark contain almost identical concentrations of glycosides, saponins, phytosterols, and flavonoids, the compounds most often found in plant extracts. This pharmacological study employed two extracts[1-5]. You can see the results in Table 3.

3.4 Acute Toxicity Study

To evaluate a medicine's therapeutic index and guarantee its safety in vivo, acute toxicity tests are

Table 3. Preliminary phytochemical studies of stem bark of *N. arbor-tristis*

Constituents	Tests	Pet.ether extract	$CHCl_3$ Extract	Acetone extract	Ethanolic extract	Aqueous extract
Carbohydrate	Molisch's test	-	-	-	-	-
	Fehling's test	-	-	-	-	+
Glycosides	Legal's test	-	-	-	+	+
	Borntrager's test	-	-	-	+	+
	Baljet test	-	-	-	-	-
Fixed oil and fats	Spot test	-	-	-	-	-
	Saponification test	-	-	-	-	-
Proteins and amino acids	Millon's test	-	-	-	-	-
	Ninhydrin test	-	-	-	-	-
	Biuret test	-	-	-	-	-
Saponins	Foam test	-	-	+	+	+
Phenolic comp. and tannins	$FeCl_3$ test	-	+	+	+	+
	Lead acetate test	-	+	+	+	+
Phytosterols	Salkowski test	+	-	+	+	+
	Libermann Bucchard test	+	-	+	+	+
Alkaloids	Dragendorff's test	-	+	-	+	+
	Mayer's test	-	+	-	+	+
	Wagner's test	-	+	-	+	+
	Hager's test	-	+	-	+	-
Gums and mucilage	Froth test	+	-	-	-	-
	Alcoholic test	+	-	-	-	-
Flavonoids	Lead acetate test	-	+	+	+	+
	Con. H_2SO_4 test	-	+	+	+	+
	$FeCl_3$ test	-	+	+	+	+

performed. The LD50 is often determined by acute toxicity testing in experimental animals. Results from phytochemical analysis were used to narrow down the potential of *N. arbor-tristis* extracts for acute toxicity testing in accordance with OECD Guideline 423. There was no toxicity or anomalous behaviour seen in the groups of animals given increasing doses up to 5000 mg/kg, and no deaths occurred. All of the extracts were found to be non-toxic or harmless to rats, placing them in the highest safety category (>5000). The results are shown in Table 4.

3.5 Hepatoprotective Activity

3.5.1 Hepatoprotective Activity of N. arbor-tristis Stem Bark Extracts on Carbon Tetrachloride Induced Hepatotoxicity

3.5.1.1 Effect of N. arbor-tristis Stem Bark Extracts on Functional Parameters

When administered 40 mg/kg of thiopentone sodium was orally, all animal groups went to sleep. The CCl_4 group slept for much more hours per day than the control group, and it took them significantly longer

Table 4. Acute toxicity studies of extracts of stem bark of *N. arbor-tristis*

Sr. No.	No. of Animals	Extract	Dose (mg/kg)	Results
1	3	AENA	5	No death
2	3		50	No death
3	3		300	No death
4	3		2000	No death
5	3		5000	No death
6	3	AQENA	5	No death
7	3		50	No death
8	3		300	No death
9	3		2000	No death
10	3		5000	No death

LD50: 5000mg/kg; ED50: 500mg/kg

to fall asleep (in seconds). Pre-sleep administration of AENA, AQENA (500 mg/kg p.o.), and silymarin extracts significantly improved sleep efficiency in rats compared to CCl_4. Time to sleep was significantly shorter in the groups given AENA, AQENA (500 mg/kg p.o.), and silymarin than in the CCl_4 group (80.2 ±5.28 seconds vs. 125.6 ±5.26 seconds, respectively). The CCl_4 group slept for 235.8 ± 6.80 minutes, whereas the AENA group slept for 202.9 ± 4.89 minutes, the AQENA group slept for 180.3 ± 4.75 minutes, and the silymarin group slept for 149.2 ± 2.49 minutes[9-12] (Table 5).

3.5.1.2 Effect of Selected Plant Extracts on Physical Parameters

3.5.1.2.1 Liver Weight

Liver enlargement was shown by a rise in body mass in the CCl_4 group. The liver weights of the groups given silymarin, AENA, and AQENA (500 mg/kg, orally) returned to normal. The CCl_4 group had a liver weight of 9.12 ± 1.28 w/100 gm b.w., whereas the AENA group weighted of 7.98 ± 0.26, the AQENA group had a weight of 7.56 ± 0.21, and the silymarin group had a weight of 7.04 ± 1.48 w/100 gm b.w.

3.5.1.2.2 Liver Volume

In the groups of animals administered AENA, AQENA (500 mg/kg p.o.), and silymarin, liver size returned to normal. The reduced liver size attests to the protective effects of certain extracts on the liver. The CCl_4 group's liver volume was 9.52 ± 1.18 ml, whereas the AENA group's was 7.98 ± 1.26 ml, the AQENA group was 7.66 ± 0.28 ml, and the silymarin group's was 7.02 ± 1.49 ml[15-19] (Table 6).

Table 5. Effect of *N. arbor-tristis* stem bark extracts on functional parameters in CCl_4 induced hepatotoxic rats

Sr. No.	Treatment/Dose	Onset of sleep(Sec.)	Duration of sleep (Min.)
1	Normal	170.0 ± 2.06	110.2 ± 2.80
2	Induced (CCl_4)	80.2 ± 5.28*	235.8 ± 6.80*
3	Standard (Silymarin)	156.2 ± 3.48***	149.7 ± 2.49***
8	AENA (500mg/kg)	125.6 ± 5.26**	202.9 ± 4.89**
9	AQENA (500mg/kg)	141.8 ± 4.81***	180.3 ± 4.75***

Table 6. Effect of *N. arbor-tristis* stem bark extracts on physical parameters in CCl_4 induced hepatotoxic rats

Sr. No.	Treatment/Dose	Liver weight (wt./100gm b.w)	Liver Volume
1	Normal	6.84 ± 0.06	6.85 ± 0.07
2	Induced (CCl_4)	9.12 ± 1.28*	9.52 ± 1.18*
3	Standard (Silymarin)	7.04 ± 1.48***	7.02 ± 1.49***
8	AENA (500mg/kg)	7.98 ± 0.26**	7.98 ± 1.26**
9	AQENA (500mg/kg)	7.56 ± 0.21***	7.66 ± 0.28***

3.5.1.3 Effect of N. arbor-tristis Stem Bark Extracts on Serum Marker Enzyme Levels of Carbon Tetrachloride Induced Hepatotoxic Rats

Hepatotoxic animals had dramatically increased levels of marker enzymes in their blood, including SGPT, SGOT, and ALP. Hepatotoxicity was mitigated with pretreatment with AENA, AQENA (500 mg/kg, p.o.), and silymarin (25 mg/kg) by reducing systemic enzyme levels. Treatment with AENA, AQENA (500 mg/kg, p.o.), and silymarin reduced SGPT levels from 128.18 ± 7.24 U/L in the CCl_4-induced hepatotoxic group to 94.90 ± 2.44, 72.18 ± 8.20, and 65.06 ± 6.41 U/L, respectively. The SGOT level was 272.8 ± 8.24 U/L in the CCl_4-induced hepatotoxic group. The values were 192.42 ± 7.24 U/L in the AENA group, 181.04 ± 9.10 U/L in the AQENA group, and 170.16 ± 8.17 U/L in the silymarin group. The CCl_4 group had ALP levels of 280.42 ± 6.46 U/L, the AENA group of 248.74 ± 8.52 U/L, the AQENA (500 mg/kg, p.o.) group of 210.18 ± 8.26 U/L, and the silymarin group of 198.20 ± 8.27 U/L[21-23] (Table 7).

3.5.1.4 Effect of N. arbor-tristis Stem Bark Extracts on Biochemical Parameters Carbon Tetrachloride Induced Hepatotoxic Rats

Total bilirubin levels increased and total protein levels decreased after CCl_4 administration in mice (Table 8). Total protein and total bilirubin levels were significantly improved in the groups who received silymarin, AENA, or AQENA (500 mg/kg p.o.).

3.5.2 Hepatoprotective Activity of N. Arbor-tristis Stem Bark Extracts on Paracetamol Induced Hepatotoxic Rats

3.5.2.1 Effect of Selected Plant Extracts on Functional Parameters

When administered 40 mg/kg of thiopentone sodium was orally, all animal groups went to sleep. Mice administered paracetamol slept later (in seconds) and longer (in minutes) than their untreated counterparts (min). Animals administered extracts of AENA, AQENA (500 mg/kg, p.o.), and silymarin fell asleep and woke up much quicker than paracetamol-treated rats (Table 9).

Table 7. Effect of *N. arbor-tristis* stem bark extracts on serum marker enzyme levels of carbon tetrachloride induced hepatotoxic rats

Sr. No.	Treatment/Dose	SGPT U/L	SGOT U/L	ALP U/L
1	Normal	62.0 ± 3.71	168.04 ± 2.80	190.0 ± 8.01
2	Induced (CCl_4)	128.18 ± 7.24*	272.8 ± 8.24*	280.42 ± 6.46*
3	Standard (Silymarin)	65.06±6.41**	170.16±8.17**	198.20±8.27**
8	AENA (500mg/kg)	94.90 ± 2.44**	192.42 ± 7.24**	248.74 ± 8.52**
9	AQENA (500mg/kg)	72.18±8.20**	181.04±9.10**	210.18±8.26**

Table 8. Effect of *N. arbor-tristis* stem bark extracts on biochemical parameters carbon tetrachloride induced hepatotoxic rats

Sr. No.	Treatment/Dose	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
1	Normal	0.38 ± 0.06	9.57 ± 0.24
2	Induced (CCl_4)	9.20 ± 0.24*	6.02 ± 1.46*
3	Standard (Silymarin)	0.54 ± 0.20***	9.24 ± 1.26***
4	AEAC (500mg/kg)	0.70 ± 0.02**	7.22 ± 1.12*
5	AQEAC (500mg/kg)	0.62 ± 0.42***	7.28 ± 0.42**
6	EESV (500mg/kg)	0.64 ± 0.66**	8.26 ± 0.20**
7	AQESV (500mg/kg)	0.58 ± 0.60***	9.16 ± 1.43***
8	AENA (500mg/kg)	0.67 ± 0.08**	7.25 ± 1.46**
9	AQENA (500mg/kg)	0.60 ± 0.60***	8.71 ± 1.24***

Table 9. Effect of *N. arbor-tristis* stem bark extracts on functional parameters in paracetamol induced hepatotoxic rats

Sr. No.	Treatment/Dose	Onset of sleep (Sec)	Duration of sleep (Min)
1	Normal	170.0 ± 2.06	110.2 ± 2.80
2	Induced (Paracetamol)	98.4 ± 6.28*	255.8 ± 5.90*
3	Standard (Silymarin)	176.6 ± 4.48***	140.2 ± 4.49***
8	AENA (500mg/kg)	135.1 ± 6.20**	202.9 ± 5.99**
9	AQENA (500mg/kg)	149.8 ± 5.81***	192.3 ± 4.55***

Table 10. Effect of *N. arbor-tristis* stem bark extracts on physical parameters in paracetamol induced hepatotoxic rats

Sr. No.	Treatment/Dose	Liver weight (wt./100gm b.w)	Liver Volume
1	Normal	6.84 ±0.06	6.97 ± 0.05
2	Induced (Paracetamol)	8.84 ± 0.48*	9.02 ± 0.49*
3	Standard (Silymarin)	7.02 ± 0.46***	7.36 ± 0.49***
8	AENA (500mg/kg)	7.62 ± 0.66**	7.87 ± 0.68**
9	AQENA (500mg/kg)	7.38 ± 0.80***	7.59 ± 0.83***

Table 11. Effect of *N. arbor-tristis* stem bark extracts on serum enzyme parameter in paracetamol induced hepatotoxic rats

Sr. No.	Treatment/Dose	SGPT U/L	SGOT U/L	ALP U/L
1	Normal	62.0 ± 3.71	168.04 ± 2.80	190.0 ± 8.01
2	Induced (Paracetamol)	154.8 ± 8.64*	248.4 ± 9.24*	360.20±8.82*
3	Standard (Silymarin)	86.86±8.63***	176.16±8.17***	166.35±4.27***
8	AENA (500mg/kg)	94.90 ± 4.62**	208.48 ± 8.64**	202.20 ± 4.52**
9	AQENA (500mg/kg)	92.24 ± 8.24***	186.48 ± 8.52***	193.0 ± 6.14***

3.5.2.2 Effect of N. arbor-tristis Stem Bark Extracts on Physical Parameters

3.5.2.2.1 Liver Weight

Liver weight increased in response to paracetamol administration in rats. The liver weights of the groups given silymarin, AENA, and AQENA (500 mg/kg, orally) returned to normal (Table 10). The CCl_4 group had a liver weight of 9.12 ±1.28 w/100 gm b.w., whereas the AENA group weighted of 7.98 ±0.26, the AQENA group had a weight of 7.56 ±0.21, and the silymarin group had a weight of 7.04 ± 1.48 w/100 gm b.w.

3.5.2.2.2 Liver Volume

When mice were given paracetamol, their livers grew a lot. However, when they were given AENA, AQENA (500 mg/kg p.o.), and silymarin, their livers went back to normal. Some extracts have hepatoprotective properties because they cause the liver to shrink. The liver volume of the CCl_4 group was 9.52 ±1.18 ml, while the liver volumes of the AENA, AQENA (500 mg/kg, Pu), and silymarin groups were all 7.98 ± 1.26 ml (Table 10).

3.5.2.3 Effect of N. arbor-tristis Stem Bark Extracts on Serum Marker Enzyme Levels of Paracetamol Induced Hepatotoxic Rats

Blood enzyme levels of SGOT, SGPT, and ALP rose dramatically in rats with hepatotoxicity. The increased levels of blood marker enzymes were reduced after pretreatment with AENA, AQENA (500 mg/kg, p.o.), and silymarin (25 mg/kg) to prevent hepatotoxicity (Table 11).

3.5.2.4 Effect of N. arbor-tristis Stem Bark Extracts on Biochemical Parameter in Paracetamol Induced Hepatotoxic Rats

Total bilirubin and total protein levels increased and decreased, respectively, in the paracetamol-treated groups. Total protein and total bilirubin were significantly increased and decreased, respectively, in the silymarin, AENA, and AQENA (500 mg/kg p.o.) treated groups (Table 12).

3.5.3 Effect of N. arbor-tristis Stem Bark Extracts on Ethanol Induced Hepatotoxicity

3.5.3.1 Effect of N. arbor-tristis Stem Bark Extracts on Functional Parameters

When administered 40 mg/kg of thiopentone was sodium orally, all animal groups went to sleep. The ethanol group of rats slept far longer and more soundly than the control group rats (min). AENA, AQENA (500 mg/kg, p.o.), and silymarin extracts significantly improved sleep and wake times compared to ethanol alone in rats[15,18,20-23] (Table 13).

3.5.3.2 Effect of N. arbor-tristis Stem Bark Extracts on Physical Parameters

3.5.3.2.1 Liver Weight

An increase in liver weight was seen in ethanol-treated rats. The liver weights of the groups given silymarin, AENA, and AQENA (500 mg/kg, p.o.) returned to normal.

3.5.3.2.2 Liver Volume

Mice given AENA, AQENA (500 mg/kg p.o.), and silymarin had a considerably smaller liver volume compared to mice given ethanol. Liver volume loss is evidence that certain extracts have hepatoprotective properties (Table 14).

Table 12. Effect of *N. arbor-tristis* stem bark extracts on biochemical parameter in paracetamol induced hepatotoxic rats

Sr. No.	Treatment/Dose	Total Bilirubin mg/dl	Total Protein gm/dl
1	Normal	0.38 ± 0.06	9.57 ± 0.24
2	Induced(Paracetamol)	5.42 ± 0.11*	5.42 ± 1.46*
3	Standard (Silymarin)	0.45 ± 0.82***	9.21 ± 1.26***
8	AENA (500mg/kg)	1.56 ± 0.20**	8.14 ± 0.46**
9	AQENA (500mg/kg)	0.52 ± 0.48***	8.28 ± 0.84***

Table 13. Effect of *N. arbor-tristis* stem bark extracts on functional parameters in ethanol induced hepatotoxic rats

Sr. No.	Treatment/Dose	Onset of sleep (Sec)	Duration of sleep (Min)
1	Normal	170.0 ± 2.06	110.2 ± 2.80
2	Induced (Ethanol)	95.4 ± 4.28*	248.4 ± 4.90*
3	Standard (Silymarin)	172.6 ± 4.98***	122.2 ± 4.89***
8	AENA (500mg/kg)	142.5 ± 4.20**	188.9 ± 5.99**
9	AQENA (500mg/kg)	154.8 ± 5.10***	158.8 ± 4.85***

Table 14. Effect of *N. arbor-tristis* stem bark extracts on physical parameters in ethanol induced hepatotoxic rats

Sr. No.	Treatment/Dose	Liver weight (gm)	Liver Volume (ml)
1	Normal	6.84 ± 0.06	6.97 ± 0.08
2	Induced (Ethanol)	8.24 ± 0.28*	8.38 ± 0.29*
3	Standard (Silymarin)	7.06 ± 0.48***	7.36 ± 0.48***
8	AENA (500mg/kg)	7.92 ± 0.96**	8.07 ± 0.98**
9	AQENA (500mg/kg)	7.48 ± 0.80***	7.59 ± 0.82***

Table 15. Effect of N. arbor-tristis stem bark extracts on serum marker enzyme levels in ethanol induced hepatotoxic rats

Sr. No.	Treatment/Dose	SGPT U/L	SGOT U/L	ALP U/L
1	Normal	62.0 ± 3.71	168.04 ± 2.80	190.0 ± 8.01
2	Induced (Ethanol)	98.75 ± 8.86*	258.42 ± 4.24*	244.76 ± 8.82*
3	Standard(Silymarin)	63.76±4.63**	176.28±8.47**	194.27±4.27**
8	AENA (500mg/kg)	69.84 ± 8.26**	189.82 ± 7.20**	200.28 ± 8.62**
9	AQENA 500mg/kg	67.82±6.24***	183.30±4.52***	197.41±8.14***

Table 16. Effect of *N. arbor-tristis* stem bark extracts on biochemical parameter in ethanol induced hepatotoxic rats

Sr. No.	Treatment/Dose	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
1	Normal	0.38 ± 0.06	9.57 ± 0.24
2	Induced (Ethanol)	6.42 ± 0.66*	5.40 ± 1.46*
3	Standard (Silymarin)	0.45 ± 0.82***	9.81 ± 1.26***
8	AENA (500mg/kg)	0.57 ± 0.28**	8.34 ± 1.26**
9	AQENA (500mg/kg)	0.52 ± 0.48***	8.82 ± 1.64***

Table 17. Effect of *N. arbor-tristis* stem bark extracts on functional parameters in RIF+INH induced hepatotoxic rats

Sr. No.	Treatment/Dose	Onset of sleep (Sec)	Duration of sleep (Min)
1	Normal	170.0 ± 2.06	110.2 ± 2.80
2	Induced (RIF+INH)	87.4 ± 4.88*	240.2 ± 4.90*
3	Standard (Silymarin)	178.5 ± 4.28***	122.2 ± 4.99***
8	AENA (500mg/kg)	142.1 ± 5.20**	192.2 ± 5.09**
9	AQENA (500mg/kg)	155.2 ± 5.21***	168.5 ± 4.56***

3.5.3.3 Effect of selected N. arbor-tristis Stem Bark Extracts on Serum Marker Enzyme Levels of Ethanol Induced Hepatotoxic Rats

Hepatotoxic animals had significantly elevated levels of the blood enzymes SGOT, SGPT and ALP. Hepatotoxicity was avoided when animals were pretreated with either AENA or AQENA (500 mg/kg, p.o.) or silymarin (25 mg/kg), both of which reduced elevated levels of blood marker enzymes (Table 15).

3.5.3.4 Effect of N. arbor-tristis Stem Bark Extracts on Biochemical Parameters Ethanol Induced Hepatotoxic Rats

The total bilirubin and total protein levels were both drastically altered in the ethanol-treated groups. The total bilirubin levels of the AENA, AQENA (500 mg/kg, p.o.), and silymarin-treated groups were significantly lower, while the total protein levels were higher (Table 16).

3.5.4 Effect of N. arbor-tristis Stem Bark Extracts on Rif+Inh Induced Hepatotoxic Rats

3.5.4.1 Effect of N. arbor-tristis Stem Bark Extracts on Functional Parameters

All of the animal groups fell asleep after receiving an intravenous dose of thiopentone sodium (40 mg/kg). RIF+INH-treated rats significantly lagged behind the control group in both sleep onset (measured in seconds) and total time spent asleep (min). Pretreatment with AENA, AQENA (500 mg/kg p.o.), and silymarin extracts substantially shortened the time it took mice to go asleep and wake up from sleep compared to rats pretreated with RIF+INH (Table 17).

Table 18. Effect of *N. arbor-tristis* stem bark extracts on physical parameters in RIF+INH induced hepatotoxic rats

Sr. No.	Treatment/Dose	Liver weight (gm)	Liver Volume (ml)
1	Normal	6.84 ± 0.06	6.97 ± 0.08
2	Induced (RIF+INH)	8.48 ± 0.28*	8.60 ± 0.26*
3	Standard (Silymarin)	7.02 ± 0.48***	7.36 ± 0.49***
8	AENA (500mg/kg)	7.40 ± 0.86**	7.57 ± 0.87**
9	AQENA (500mg/kg)	7.30 ± 0.80***	7.50 ± 0.83***

Table 19. Effect of *N. arbor-tristis* stem bark extracts on serum enzyme parameter on RIF+INH induced hepatotoxic rats

Sr. No.	Treatment/Dose	SGPT U/L	SGOT U/L	ALP U/L
1	Normal	62.0 ± 3.71	168.04 ± 2.80	190.0 ± 8.01
2	Induced (RIF+INH)	174.41 ± 8.24*	368.72 ± 8.24*	343.44 ± 7.56*
3	Standard (Silymarin)	65.52±3.41***	170.80±4.67***	200.29±8.23***
8	AENA (500mg/kg)	86.20 ± 8.24**	200.22 ± 4.24**	226.20 ± 8.88**
9	AQENA (500mg/kg)	78.22 ± 4.82***	186.48 ± 8.52***	218.0 ± 8.48***

Table 20. Effect of *N. arbor-tristis* stem bark extracts on biochemical parameters rifampicin isoniazid induced hepatotoxic rats

Sr. No.	Treatment/Dose	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
1	Normal	0.38 ± 0.06	9.57 ± 0.24
2	Induced (RIF+INH)	6.76 ± 8.04*	5.2 ± 0.16*
3	Standard (Silymarin)	0.42 ± 2.68***	9.60 ± 4.80***
8	AENA (500mg/kg)	0.58 ± 4.58**	6.84 ± 8.66**
9	AQENA(500mg/kg)	0.54 ± 4.58***	8.14 ± 9.44***

3.5.4.2 Effect of N. arbor-tristis Stem Bark Extracts on Physical Parameters

3.5.4.2.1 Liver Weight

Liver enlargement was seen in RIF+INH-treated animals as shown by a weight gain in the organ. The liver weight was brought significantly closer to normal after treatment with AENA, AQENA (500 mg/kg, p.o.), and silymarin.

3.5.4.2.2 Liver Volume

The liver size of rats treated with RIF+INH increased dramatically, but after receiving AENA, AQENA (500 mg/kg p.o.), and silymarin, the liver size returned to normal. A significant reduction in liver size is evidence that certain extracts have liver-protective effects (Table 18).

3.5.4.3 Effect of N. arbor-tristis Stem Bark Extracts on Serum Marker Enzyme Levels on RIF+INH Induced Hepatotoxic Rats

Blood enzyme levels of SGOT, SGPT, and ALP rose dramatically in rats with hepatotoxicity. The increased levels of blood marker enzymes were reduced after pretreatment with AENA, AQENA (500 mg/kg, p.o.), and silymarin (25 mg/kg) to prevent hepatotoxicity (Table 19).

3.5.4.4 Effect of N. arbor-tristis Stem Bark Extracts on Biochemical Parameters RIF+INH Induced Hepatotoxic Rats

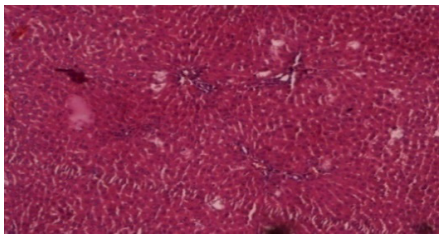
In RIF+INH-treated groups, total bilirubin was substantially higher, whereas total protein was significantly lower (Table 20). Total protein and total bilirubin were significantly

increased and decreased, respectively, in the silymarin, AENA, and AQENA (500 mg/kg p.o.) treated groups[23-25].

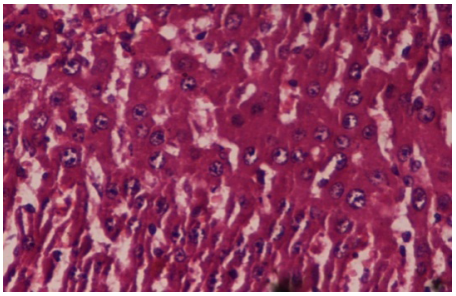
3.6 Histopathology

Biochemical investigation of a subset of extracts indicated that they have hepatoprotective properties; this finding was corroborated by histopathological studies. Some extracts may be utilised to cure histological abnormalities brought on by hepatotoxicant such CCl_4, paracetamol, ethanol, and RIF+INH[26-27]. The outcome was shown in the Figures 3-6.

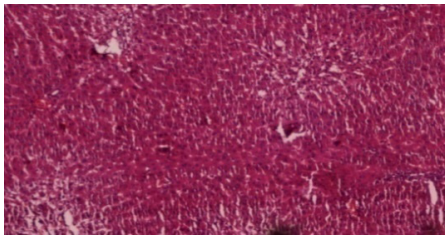
2.6.1 Effect of N. arbor-tristis Stem Bark Extracts on Histopathological Diagram of Liver Tissue in CCl_4 Induced Hepatotoxic Rats



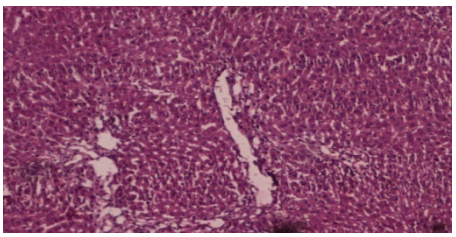
Normal: The layout is tried-and-true. The central veins, sinuses, and portal triads are all healthy. Oval to spherical nuclei and a considerable amount of cytoplasm define hepatocytes. No periportal irritation exists.



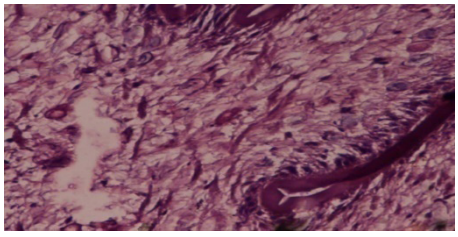
CCl_4 Induced: The building is now smaller. The sinusoids, portal triads, and central veins all seem to be blocked. Hepatocytes contain very little cytoplasm and circular or oval nuclei that have suffered a feathery degeneration. There is anger and tension in the port area.



Silymarin (25 mg/kg): Hepatocyte size and the portal area were both normal. Hepatotoxicity and the expansion of fibrous connective tissue are hardly perceptible. Maximum regenerative capacity.



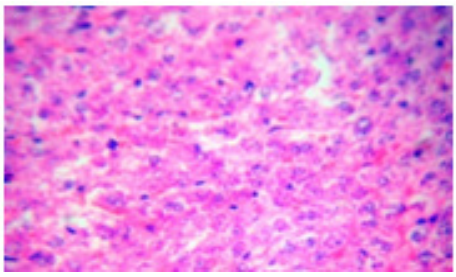
AENA (500mg/kg): The veins in this area are more prominently central. Inflammation and necrosis around a port are characterized by the presence of mononuclear cells.



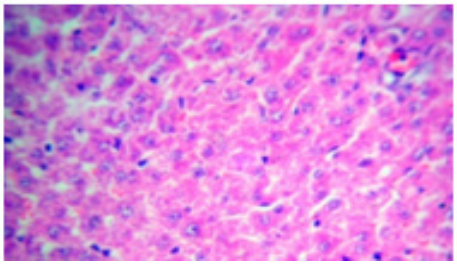
AQENA (500mg/kg): The central veins are somewhat enlarged and swollen. Healthy hepatocytes. It seems like we have a nice, neat triangle of portals.

Figure 3. Effect of *N. arbor-tristis* stem bark extracts on histopathological diagram of liver tissue in CCl_4 induced hepatotoxic rats.

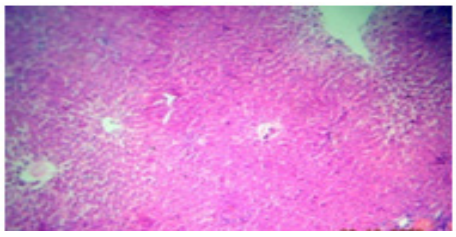
2.6.1.1 Effect of N. arbor-tristis Stem Bark Extracts on Histopathological Diagram of Liver Tissue in Paracetamol Induced Hepatotoxic Rats



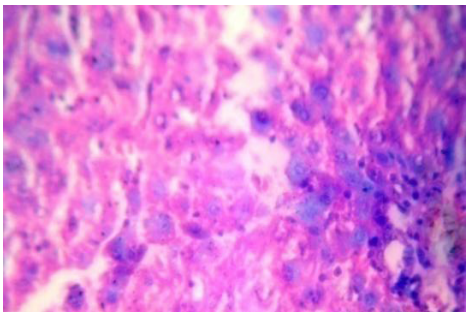
Normal: The layout is tried-and-true. The central veins, sinuses, and portal triads are all healthy. The nuclei of hepatocytes are oblong to circular and have very little cytoplasm. No periportal irritation exists.



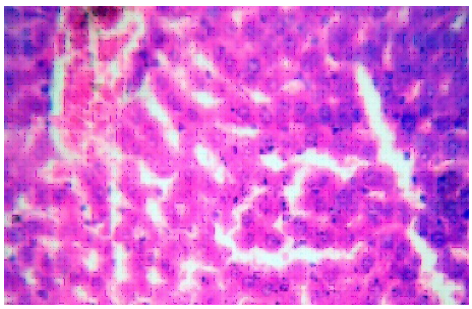
Paracetamol Induced: Swollen and enlarged central veins are present. Hepatocytes experience a kind of cellular ageing known as feathery senescence. The portal triads' minor periportal inflammation is caused by lymphocytes.



Silymarin (25 mg/kg): Major veins seem to be in good condition. Hepatocytes that are feathering show signs of ageing. The portal triads' minor periportal inflammation is caused by lymphocytes.



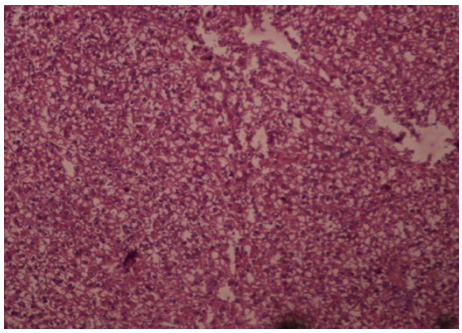
AENA (500mg/kg): Hepatocyte nuclei are a little bit bigger, shaped differently, and have a lot of colour. In the portal triads, there is a small amount of inflammation in the Peri portal area that is caused by lymphocytes. The important veins are in good shape.



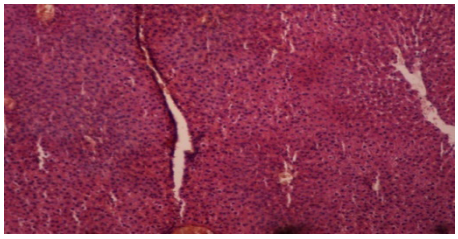
AQENA (500mg/kg): Hyper chromatic, pleomorphic, and somewhat bigger nuclei are characteristics of hepatocytes. The portal triads show signs of mild inflammation involving lymphocytes at the Peri portal level. The main veins are functioning normally.

Figure 4. Effect of *N. arbor-tristis* stem bark extracts on histopathological diagram of liver tissue in paracetamol induced hepatotoxic rats.

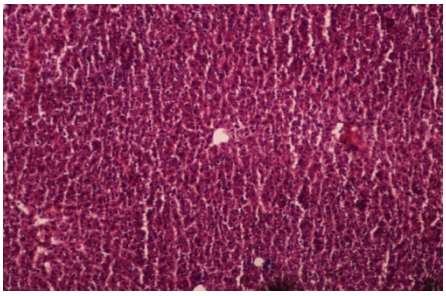
2.6.1.2 *Effect of N. arbor-tristis Stem Bark Extracts on Histopathological Diagram of Liver Tissue in Rif+Inh Induced Hepatotoxic Rats*



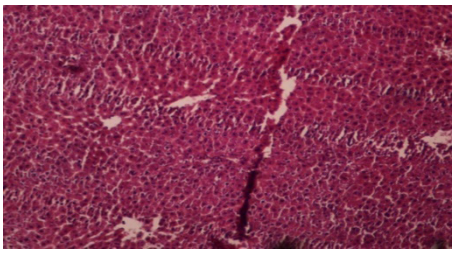
Normal: The layout is tried-and-true. The central veins, sinuses, and portal triads are all healthy. Oval to spherical nuclei and a considerable amount of cytoplasm define hepatocytes. No periportal irritation exists.



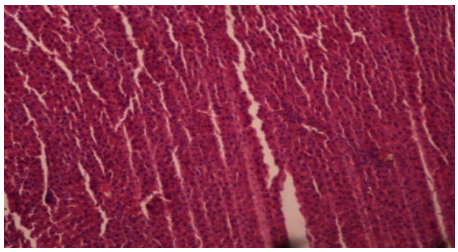
Rifampicin induced: The central veins may exhibit some dilatation and congestion. Hepatocyte steatosis and degeneration have undergone changes. The portal tracts had significant necrosis.



Silymarin (25mg/kg): The slice has wide, thick core veins that are large in size. Hepatocytes are helpful in general. The triads in the gates seem to follow a pattern. Damage to the liver and the formation of fibrous connective tissue are both limited. Highest chance of recovery.



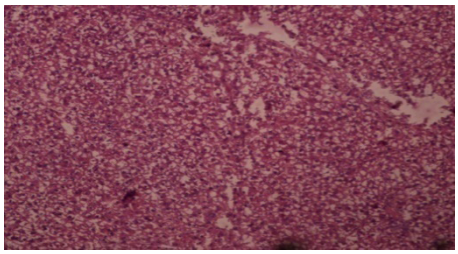
AENA (500mg/kg): Less central necrosis and increased regeneration.



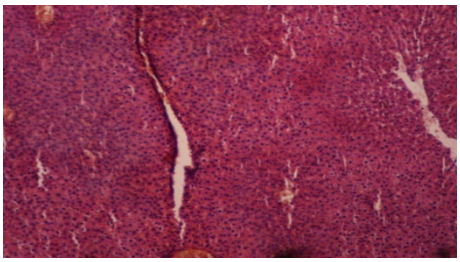
AQENA (500mg/kg): The heart's main veins seem to be healthy. Hepatocytes have a little quantity of cytoplasm, but their larger, pleomorphic nuclei are noticeable. Triads that act as gateways ordinarily.

Figure 5. Effect of *N. arbor-tristis* stem bark extracts on histopathological diagram of liver tissue in RIF+INH induced hepatotoxic rats.

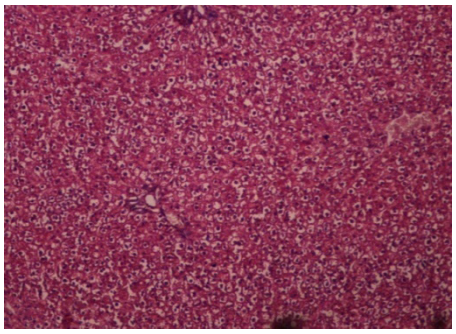
2.6.1.3 Effect of N. arbor-tristis Stem Bark Extracts on Histopathological Diagram of Liver Tissue in RIF+INH Induced Hepatotoxic Rats



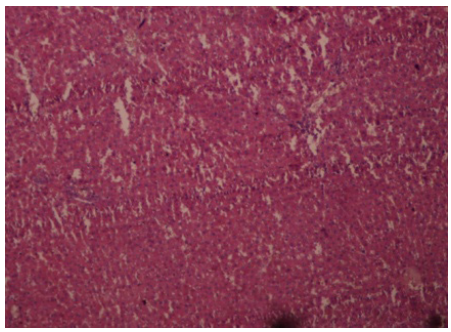
Normal: The design is a typical one. All of the sinusoids, central veins, and portal triads work well. Hepatocytes have a small amount of cytoplasm and nuclei that are oval to round. There is no inflammation around the port.



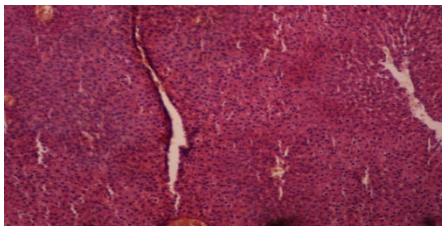
Rifampicin Induced: There is some minor congestion and dilatation in the central veins. There have been shifts in hepatocyte steatosis and degeneration. Significant necrosis was seen in the portal tracts.



Silymarin (25mg/kg): Veins in the segment's center may be observed to be enlarged and densely packed. Hepatocytes are a healthy cell type. It seems that the triads in the portals are in proper sequence. Fibrous connective tissue development and hepatotoxicity are both minimal. The greatest renewal occurs.



AENA (500mg/kg): The design is conventional. Appropriate amounts of cytoplasm and circular nuclei characterize healthy-appearing hepatocytes. Several mitotic figures may be seen in hepatocytes that are undergoing regeneration. There are normal-looking portal tracts



AQENA (500mg/kg): The veins in the middle of the body have some mild swelling and enlargement. Hepatocytes are in good shape. It looks like the portal triads are in order.

Figure 6. Effect of *N. arbor-tristis* stem bark extracts on histopathological diagram of liver tissue in RIF+INH induced hepatotoxic rats.

4. Summary and Conclusion

The extracts utilised in this investigation significantly mitigated the increases in protein and the aforementioned enzymes. Now we know the extracts have liver-saving effects. Histopathological data also demonstrated the significant effects of AENA, AQENA, and silymarin, thus bolstering the validity of this research. Liver cytoarchitecture in animals treated with hepatotoxicant is drastically altered. The results for those with severe liver issues are consistent. Mice who were administered either silymarin (25 mg/kg orally) or AQENA (500 mg/kg) as part of the AENA experiment had minor liver alterations, but their liver cytoarchitecture was unaffected. There was also evidence of hepatocyte regeneration, suggesting hepatoprotective effects. It was concluded that both AENA and AQENA had hepatoprotective properties, lending credence to their traditional uses from a scientific standpoint. Marker enzyme changes in the blood, alterations in physical measures and functional parameters, and histological analyses corroborate this finding.

5. References

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