PHARMACOLOGICAL EVALUATION AND HEPATOPROTECTIVE ACTIVITY OF *ADINA CORDIFOLIA* LEAVES AGAINST HEPATOTOXICITY IN RATS

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Abstract:

Adina cordifolia, a member of the Rubiaceae family, was investigated for its hepatoprotective properties against Wister rats that had experienced liver damage brought on by ethanol (AEAC) and its aqueous extract (AQEAC). It was discovered that AEAC and AQEAC, at a dosage of 500 mg/kg body weight, had hepatoprotective effects by dramatically reducing the levels of serum SGPT, SGOT, alkaline phosphate, and total bilirubin as well as significantly raising the levels of total protein. Histopathological examinations of liver tissue provided further evidence for the hepatoprotective action. Since blood samples from rats given ethanol treatment showed a significant increase in serum enzyme activities, reflecting liver damage brought on by ethanol, and blood samples from animals given AEAC and AQEAC showed a significant decrease in serum markers, indicating that hepatic cells were protected from ethanol-induced hepatocellular injury. Similar to the effects of the common medication silymarin, AEAC and AQEAC were effective.

Keywords: Adina cordifolia, Pharmacological Potential, Extract, Taxonomy and Hepatoprotective properties.

1 Introduction:

Plants have been described as a source of healing for over five thousand years in ancient Chinese and Indian scriptures. Several ailments have historically been treated using plant-based medications, and this practice continues today for a variety of conditions. Many individuals have utilised medicinal plants and seen their curative effects, proving their efficacy despite our incomplete understanding of their molecular mechanisms of action. Primary health care has been substantially enhanced by the use of medicinal plants. Nonetheless, there is a widespread belief that modern pharmaceuticals pose risks to human health and the natural world. On the other hand, herbal treatments are generally accepted as safe (1-4).

The abuse of alcohol and the development of alcoholism are two of the world's most pressing social and economic issues. When a person consumes too much alcohol, harmful biochemical changes occur in the liver. Cirrhosis, alcoholic hepatitis, and steatosis are all conditions that alter the liver's size and mass as a result of chronic alcohol abuse. Family Rubiaceae is where you'll find Adina cordifolia. The caustic, bitter, and aromatic bark of many plants endemic to Sri Lanka, central, and southern India, together referred to as "Haldu," has been used for centuries for its aphrodisiac, tonic, and vulnerary properties. The growing number of specialized facilities that sustain human life presents new challenges, but pharmacists and doctors have improved their ability to address these issues as they have learned more about the historical development of ideas surrounding the use of medicinal plants and the evolution of consciousness. There are over 7,000 different species of therapeutic plants known today (5-8).

Plants have an essential role in medicine due to the presence of chemical substances that have distinct effects on the functioning of the human body. Biological properties against cancer, germs, ulcers, liver damage, inflammation, diabetes, amoebas, and discomfort have been attributed to certain sections. Historically, this plant has been used to treat a wide variety of medical conditions, including but not limited to: rheumatism; digestive issues; headaches; the common cold;

toothaches; fever; pain and swelling; bacterial infections; urinary issues; conjunctivitis; and infertility (9-12).

2 Materials and Methods:

2.1 Procurement and Authentication of the Plan

The leaves of the Adina cordifolia plant were identified and validated by Dr. S. K. Billore, professor and head of the college of plant and environmental management at Vikram University in Ujjain, Madhya Pradesh. Located in Ujjain, Madhya Pradesh, the Department of Pharmacognosy at the Mahakal Institute of Pharmaceutical Sciences received the voucher specimen. (MIPS/S/005) and (MIPS/N/012/2010)

2.2 Preparation of extracts of *Adina cordifolia*

This essence originated from the leaves of the Adina cordifolia shrub. The plant's leaves were gathered, sun-dried, and then ground into a coarse powder in a mechanical grinder. The powder was sieved through a No. 40 mesh screen and then placed in an airtight container.

Figure 2.1 Leaves of *Adina cordifolia*

2.3 Physico chemical evaluation

The powder of dried and preserved plant leaves was subjected to standard techniques for the aim of assessing various physicochemical parameters (13-15).

2.3.1 Determination of ash values

Finding low-quality products, expired medications, sand, and other earthy substances may be accomplished by monitoring ash levels. The chemical components may also be determined by using acid- and water-soluble ash, respectively.

2.3.2 Determination of extractive values

During 24 hours, 100 cc of solvent (petroleum ether, chloroform, acetone, ethanol, and water) was combined with air-dried, crushed medication. The flask was shaken often for the first six hours, and then let to stand for the remaining 18 hours. After then, the liquid was filtered rapidly to prevent the alcohol from evaporating. A flat-bottomed shallow dish was used to heat a 25 ml sample of the filtrate to 105℃. After then, the dish's weight was recorded. To determine how much of the extractive is water-soluble, the air-dried medication was employed as a benchmark.

2.4 Preliminary phytochemical studies (16-18)

2.4.1 Tests for carbohydrates and glycosides

The extracts were each diluted in 4 cc of distilled water and filtered before use. Various analyses were run on the filtrate to check for the presence of carbohydrates.

2.4.2 Test for alkaloids

Solvent-free alcoholic and aqueous extracts were mixed with a small amount of diluted hydrochloric acid before filtration. The presence of alkaloids in the filtrate was determined using a number of different reagents.

2.4.3 Test for proteins and free amino acids (19, 20)

For the purpose of finding functional groups in proteins and amino acids, there are six assays available. The six examinations are as follows: (1) Ninhydrin Test (2) Biuret Test (3) Xanthoproteic Test (4) Millon's Test (5) Hopkins-Cole Examination and (6) Nitroprusside Examination.

2.4.4 Test for flavonoids

The stock solution (1 mL) in a test tube was diluted by adding a few drops of a diluted NaOH solution. A bright yellow hue could be noticed in the test tube. A few drops of diluted acid rendered it Colourless, proving the existence of flavonoids.

2.4.5 Test for saponins

Put 2 ml of the extract's water solution in a test tube and mix it up. The production of steady foam indicates the presence of saponins (froth).

2.5 Pharmacological studies (21-23)

2.5.1 Animals

In order to conduct acute toxicity studies, Wistar albino female rats (weighing between 150 and 200 g) were purchased from the Central Drug Research Organization in Lucknow. They lived in polypropylene cages with free access to water and a normal rat pellet food (both provided by Hindustan Lever Ltd, Bangalore). The rodents were subjected to a light-dark cycle that repeated itself every 12 hours. The experimental techniques were reviewed and approved by the institution's animal ethics committee, and the rats fasted for at least 12 hours prior to the test. All tests were performed in the morning to comply with the CPCSEA's ethical guideline for the study of experimental pain in conscious animals and the standards for the care of laboratory animals. Rats were given medication orally using a regular orogastric cannula.

2.5.2 Chemicals

The solvents and chemicals were all of analytical grade. The common medication silymarin (25 mg/kg, b.w.) was utilised.

2.5.3 Acute toxicity studies

The determination of the fatal dosage is a step in the acute toxic category approach for evaluating acute oral toxicity. A single oral gavage dosage of the test chemical is given to fasting young adult rats. After up to 15 days of observation and weight monitoring, necropsies are done on all of the animals. Doses of 5, 50, 300, 2000, and 5000 milligramme per kilogramme of body weight were

used to administer the test drugs. The lethal dosages were discovered. In this investigation, female rats were utilised across the board, with three rats per treatment group.

2.5.4 Hepatoprotective studies (24-26)

2.5.4.1 Carbon tetrachloride (CCl_4) induced hepatotoxicity

An increase in intracellular reactive Fe^{+2} ions, aldehyde, and GSH depletion, as well as calcium sequestration, are all caused by the nascent oxygen O^- coming from lipoperoxidation, which is produced when the medication is metabolized in the endoplasmic reticulum and mitochondria. Ca^{+2} sequestrations degrade due to both direct covalent interaction and oxidative CCl_3 O^-. Aldehyde cytotoxicity by lipid peroxidation is precipitated in the absence of sequestration due to increased intercellular Ca+2, aggregation by proteolytic enzymes, and an increase in Fe^{+2} ions.

2.5.4.2 Paracetamol induced hepatotoxicity

A hepatotoxic metabolite is the molecule responsible for paracetamol-induced liver damage. Sulfate and glucuronide conjugates are the most common metabolites of paracetamol at therapeutic levels. The remaining compounds are converted into reactive intermediates and rendered harmless by linking them to glutathione. When the sulphate and glucuronide pathways are depleted, an overdose causes a greater proportion of the drug to be converted into the reactive metabolite. By supplementing the liver with glutathione-like compounds, such as acetyl cysteine, the reactive metabolite may be eliminated through the conjugation pathway. This prevents further damage to the liver and protects its cells.

2.5.4.3 Ethanol induced hepatotoxicity

There are several ways in which excessive alcohol use may damage your liver. The most prevalent outcomes are hepatitis, cirrhosis, and liver fat accumulation. Alcohol is harmful to the liver regardless of any nutritional inadequacies since it is toxic in and of itself. Even at very low doses, ethanol may accelerate the normal accumulation of fat in the liver of healthy persons. This accumulates due to a decrease in the rate of the tricarboxylic acid cycle and fat oxidation. This is in part due to the high production of NADH by alcohol and aldehyde dehydrogenases. Fibrosis occurs

as a result of tissue loss and chronic inflammation, both of which are hallmarks of alcoholic cirrhosis. Fibrous tissue has taken the place of normal liver tissue. There is some evidence that alcohol directly affects the stellate cells in the liver, leading to an accumulation of collagen near the liver's venal outlet. Excessive alcohol consumption causes stellate cells to transform into collagen-producing myofibroblasts. Mallory bodies are a hallmark of alcoholic cirrhosis and have been related to alterations in the cytokeratin intermediate cytoskeleton. There are a number of proposed molecular processes.

2.5.4.4 Rifampicin induced hepatotoxicity

While rifampicin is the drug of choice for treating TB, its use over extended periods of time has been linked to liver damage. Rifampicin causes hepatotoxicity because it competes with bilirubin for uptake by liver cells. Conjugated or unconjugated hyperbilirubinemia is common in patients with rifampicin-induced chronic hepatitis.

2.6 Assessment of hepatoprotective effect of selected plant extracts (27, 28)

The protective effects of thiopentone sodium (40 mg/kg, i.p.) were evaluated by observing the animals' sleeping habits on the last day following injection.

2.6.1 Physical parameters

2.6.1.1 Determination of liver weight

After slaughtering the animals, the livers were removed, washed in saline, and their individual weights were recorded using an electronic scale. Liver weights were reported in grammes (gm) as a percentage of total body mass.

2.6.1.2 Determination of liver volume

Each liver's displacement volume was calculated by putting it in a graduated cylinder with either distilled water or saline.

2.6.2 Biochemical parameters (29)

2.6.2.1 Effect of selected plant extracts on serum glutamate pyruvate transamainse (SGPT)

To produce pyruvate and L-glutamate, SGPT (ALT) catalysis the transfer of the amino group from L-alanine to alpha-Ketoglutarate. After this, lactate dehydrogenase converts pyruvate and NADH into lactate and NAD. Reducing absorbance at 340 nm is a side effect of converting NADH to NAD. Correlating SGPT activity with the rate of absorbance loss.

2.6.2.2 Effect of selected plant extracts on serum glutamate oxalacetate transminase (SGOT)

Oxaloacetate and L-glutamate are produced when SGOT (AST) transfers an amino group from L-aspartate to alpha-Ketoglutarate. Oxaloacetate and NADH are then converted by malate dehydrogenase into malate and NAD (MDH). The rate at which NADH is converted into NAD correlates with the efficiency of SGOT, as measured by a decrease in absorbance at 340 nm.

2.6.2.3 Effect of selected plant extracts on alkaline phosphatase (ALP)

Hydrolase enzymes known as phosphatases may hydrolyze a wide variety of organic phosphates into an alcohol and phosphate ions. The diagnostic value of both alkaline phosphatase and acid phosphatase cannot be overstated. Their reactions to acidic and alkaline conditions serve as reliable biochemical fingerprints. The activity of alkaline phosphatase is determined at a pH of 10, whereas that of acid phosphatase is determined at a pH of 5.

2.6.2.4 Estimation of serum bilirubin

When alcohol is present and protein has settled out, serum bilirubin will react with diasol regent. Alcohol facilitates the detection of both conjugated and unconjugated bilirubin. Subtracting the direct bilirubin measurement from this sum yields the amount of unconjugated bilirubin. The average adult's blood level of conjugated bilirubin is 0.25 mg/dl. Problems with hepatic absorption and conjugation of bilirubin therapy, such as Gilbert's disease, and hemolysis can contribute to elevated bilirubin levels.

2.6.2.5 Serum protein

Liver cells produce several proteins, including fibrinogen, albumin, prothrombin, alpha-1 antitrypsin, hemoglobin, ceruloplasmin, transferrin, and thrombin. When there is significant liver injury, plasma levels of these proteins decrease. Total protein levels are typically considered healthy when they fall between 5.5 and 8 gm/dL. Liver disease and severe hepatocyte destruction may lead to Hypoalbuminanemia. Liver inflammatory illnesses including cirrhosis and chronic hepatitis may lead to hyperglobulinemia over time.

2.7 Histopathological studies (30)

The livers of the animals were removed when they were slaughtered, sliced into minute pieces, and preserved by being fixed in 10% formalin for two days. The liver sample was dehydrated for three 12-hour periods with 70% to 90% isopropyl alcohol after being rinsed for 12 hours under running water to remove the formalin. The patient gets dehydrated after three to five 12-hour bouts of receiving pure alcohol. Dehydration was performed to eliminate any remaining moisture. The alcohol was removed with chloroform, and then the chloroform was eliminated with paraffin penetration. Two 15- to 20-minute applications of chloroform were employed to sanitize the area. The liver slices were processed by an automated tissue processing equipment after paraffin was applied. Vacuum-embedded L-shaped blocks were made by pouring molten hard paraffin. The liver was cut into small pieces and placed directly into the hot paraffin. The blocks were sectioned off at 5 m using a microtome. The slices were placed on a micro slide coated with egg albumin.

3 Results and Discussion

3.1 Physico chemical evaluation

The leaves of A. cordifolia were subjected to a battery of physical and chemical analyses. Total ash, acid-insoluble ash, and water-soluble ash were all employed to calculate ash values in this experiment. The high concentration of inorganic elements in A. cordifolia may be seen in the 6.5% of ash detected in its leaves. The leaves of A. cordifolia were found to contain 2.5% acid-insoluble

ash. It was thought that 1.2% of the ash in A. cordifolia leaves could dissolve in water. The results are shown in Table 3.1.

Table 3.1 Physicochemical analysis of A. cordifolia leaves

Plant Name	**Part Used**	**Types of Ash**	**Percentage of Ash(w/w)**
A. cordifolia	Leaves	Total ash	6.5
		Acid insoluble	2.5
		Water soluble	1.2

3.2 Determination of extractive value

The extracted values shown in Table 3.2 indicate that there are likely substantial levels of phytoconstituents present in the solvents.

Table 3.2 Extractive Values of *A. cordifolia* leaves

Solvent	**% Yield**
	A. cordifolia
Pet. Ether	1.67
Chloroform	2.23
Acetone	14.1
Ethanol	9.21
Aqueous	16.2

3.3 Preliminary Phytochemical Analysis

Chemical analysis revealed that several extracts included unique components, elucidating the nature of the phytoconstituents. The findings demonstrated that acetone, ethanol, and water extracts of A. cordifolia leaves contain almost identical concentrations of glycosides, saponins, phytosterols, and flavonoids, the compounds typically found in plant extracts. The results are shown in Table 3.3.

Table 3.3 Preliminary phytochemical studies of leaves of *A. cordifolia*

Constituents	**Tests**	**Pet. ether extract**	**$CHCl_3$ Extract**	**Acetone extract**	**Ethanol extract**	**Aqueous extract**
Carbohydrate	*Molisch's test*	-	-	-	+	+
	Fehling's test	-	-	-	+	+
Glycosides	*Legal's test*	-	-	-	-	-
	Borntrager's test	-	-	-	-	-
	Baljet test	-	-	-	-	-
Proteins and amino acids	*Millon's test*	-	+	-	+	+
	Ninhydrin test	-	+	-	+	+
	Biuret 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	-	-	-	-	-
Flavonoids	*Lead acetate test*	-	-	+	+	+
	Con. H_2SO_4 test	-	-	+	+	+
	FeCl3 test	-	-	+	+	+

3.4 Acute toxicity study

Acute toxicity studies are conducted to determine the efficacy and safety of a drug in humans. The LD50 is often determined by performing acute toxicity studies on experimental animals. The acute toxicity of A. cordifolia extracts prepared in acetone and water was evaluated in accordance with OECD Guideline 423. There was no mortality, toxicity, or abnormal behaviour seen in groups of rats given 5,000 mg/kg in a dose-escalating study. This indicates that all of the extracts fell into the same category that is, they were either harmless to rats or had no ill effects on them.

Table 3.4 Acute toxicity studies of extracts of *A. cordifolia*

Sr. No.	No. of Animals	Extract	Dose (mg/kg)	Results
1	3	AEAC	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	AQEAC	5	No death
7	3		50	No death
8	3		300	No death
9	3		2000	No death
10	3		5000	No death

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

3.5 Hepatoprotective activity

3.5.1 Carbon tetrachloride (CCl_4) induced hepatotoxicity

All of the animal groups went to sleep after receiving an intravenous dose of thiopentone sodium (40 mg/kg). As compared to the control group, rats given CCl4 took much longer to fall asleep and slept for significantly longer periods of time once they did (min). Animals pretreatment with AEAC, AQEAC (500 mg/kg p.o.), and silymarin exhibited substantially faster sleep start and shorter sleep duration compared to CCl4-exposed and silymarin-pretreated mice. The CCl4 group's first sleep time was 80.2 ± 5.28 sec, whereas those in the AEAC, AQEAC (500 mg/kg p.o.), and silymarin groups slept for 110.2 ± 4.48 sec and 130.8 ± 4.76 sec, respectively. The CCl4 group slept for 235.8 ± 6.80 minutes, whereas the AEAC, AQEAC (500 mg/kg, p.o.), and silymarin groups slept for 210.8 ± 5.88, 192.2 ± 5.76, and 0 minutes, respectively.

Table 3.5 Effect of extracts of *A. cordifolia* leaves on functional parameters in CCl4 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 (CCl4)	80.2 ± 5.28*	235.8 ± 6.80*
3	Standard (Silymarin)	156.2 ± 3.48***	149.7 ± 2.49***
4	AEAC (500mg/kg)	110.2 ± 4.48**	210.8 ± 5.88**
5	AQEAC (500mg/kg)	130.8 ± 4.76***	192.2 ± 5.76***

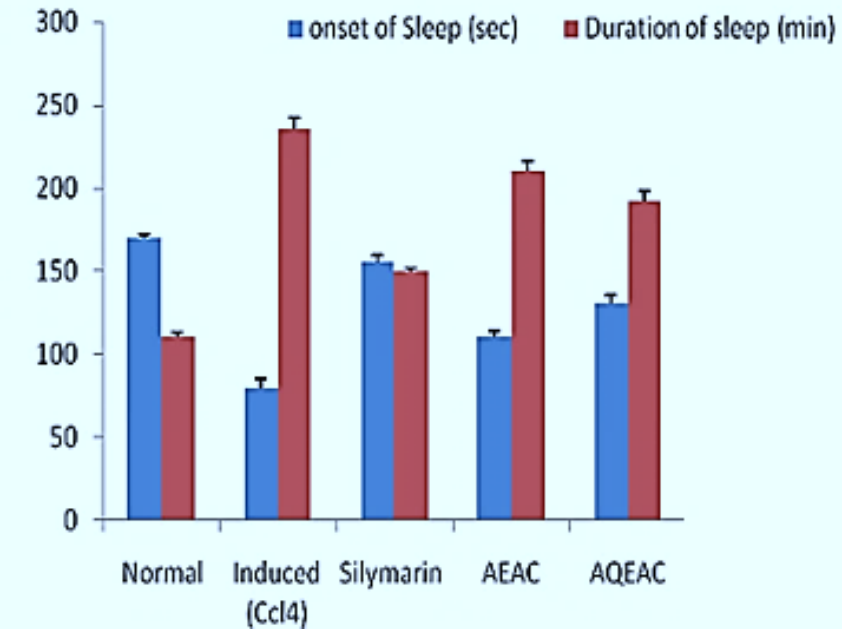


Figure 3.1 Effect of A. cordifolia leaves extracts on functional parameters in CCl4 induced hepatotoxic rats.

3.5.1.1 Effect of *A. cordifolia* leaves extracts on physical parameters

Table 3.6 Effect of *A. cordifolia* leaves extracts on physical parameters in CCl4 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 (CCl4)	9.12 ± 1.28*	9.52 ± 1.18*

3	Standard (Silymarin)	7.04 ± 1.48***	7.02 ± 1.49***
4	AEAC (500mg/kg)	8.38 ± 0.48**	8.39 ± 0.88**
5	AQEAC (500mg/kg)	7.80 ± 0.76***	7.91 ± 0.76***

Values are mean ± SEM, n = 6.

3.5.1.2 Effect of *A. cordifolia* leaves extracts on serum marker enzyme levels of carbon tetrachloride induced hepatotoxic rats

Table 3.7 Effect of *A. cordifolia* leaves 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 (CCl4)	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***
4	AEAC (500mg/kg)	98.18 ± 7.20**	231.0 ± 8.13**	260.0 ± 6.31**
5	AQEAC(500mg/kg)	89.56±5.61***	188.0 ± 8.66***	240.15±6.28***

3.5.1.3 Effect of *A. cordifolia* leaves extracts on biochemical parameter

Table 3.8 Effect of *A. cordifolia* leaves 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(CCl4)	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**

3.5.2 Hepatoprotective activity of *A. cordifolia* leaves extracts on paracetamol induced hepatotoxic rats

When administered 40 mg/kg thiopentone sodium orally, all animal groups went to sleep. Mice administered paracetamol slept later (in seconds) and longer (in minutes) than their untreated counterparts (min). Extracts of AEAC, AQEAC (500 mg/kg, p.o.), and silymarin caused substantially quicker sleep onset and recovery compared to paracetamol-treated rats.

Table 3.9 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	121.5 ± 4.80**	228.2 ± 5.02**
5	AQEAC (500mg/kg)	140.8 ± 5.76***	199.2 ± 5.96***

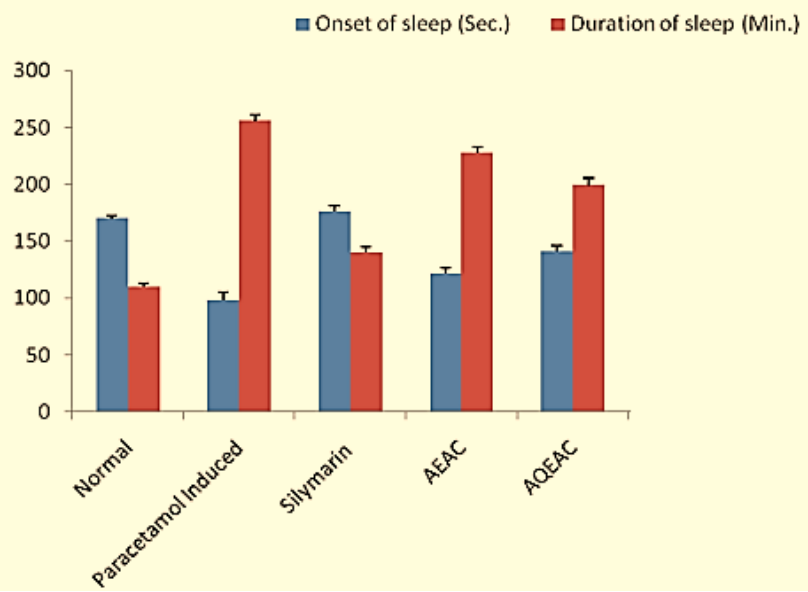


Figure 3.2 Effect of *A. cordifolia* leaves extracts on functional parameters in paracetamol induced hepatotoxic rats

3.5.2.1 Effect of *A. cordifolia* leaves extracts on physical parameters

Table 3.10 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	8.68 ± 1.26**	8.89 ± 1.28**
5	AQEAC(500mg/kg)	8.10 ± 0.86***	8.33 ± 0.88***

3.5.2.2 Effect of *A. cordifolia* leaves extracts on serum marker enzyme levels of paracetamol induced hepatotoxic rats

Table 3.11 Effect of *A. cordifolia* leaves extracts on serum enzyme parameter in paracetamol induced hepatotoxic rats.

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***
4	AEAC (500mg/kg)	131.28±8.84**	245.28 ±8.44**	260.0 ± 7.89**
5	AQEAC (500mg/kg)	120.21±4.76***	198.0 ± 9.46***	200.22±8.66***

3.5.2.3 Effect of *A. cordifolia* leaves extracts on biochemical parameter in paracetamol induced hepatotoxic rats.

Table 3.12 Effect of *A. cordifolia* leaves extracts on biochemical parameter

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***
4	AEAC (500mg/kg)	0.68 ± 0.62**	6.28 ± 0.12**
5	AQEAC (500mg/kg)	0.62.0 ± 0.58***	8.18 ± 1.48***

3.5.3 Effect of selected plant extracts on ethanol induced hepatotoxicity

When administered 40 mg/kg thiopentone sodium orally, all animal groups went to sleep. Ethanol-treated rats slept much longer and more soundly than their control group counterparts (min). Animals administered AEAC, AQEAC (500 mg/kg p.o.), or silymarin extracts went to sleep substantially sooner and slept for much shorter time than those given ethanol first.

Table 3.13 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	128.1 ± 4.02**	218.2 ± 4.68**
5	AQEAC (500mg/kg)	148.8 ± 4.76***	181.6 ± 4.90***

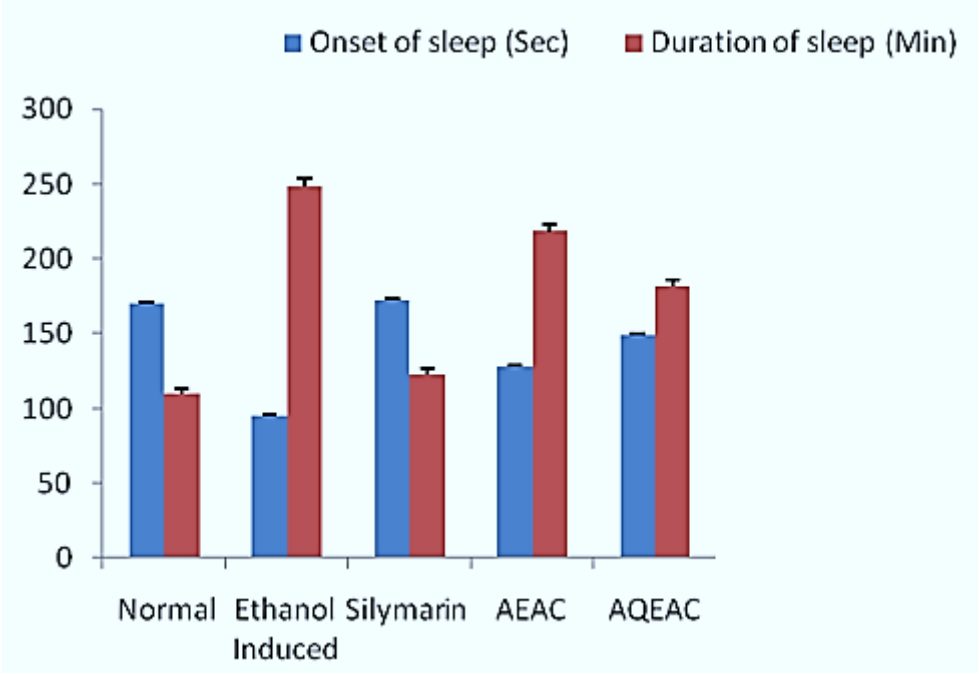


Figure 3.3 Effect of selected *A. cordifolia* leaves extracts on functional parameters in ethanol induced hepatotoxic rats.

3.5.3.1 Effect of *A. cordifolia* leaves extracts on physical parameters

Table 3.14 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	8.10 ± 1.48**	8.29 ± 1.46**
5	AQEAC (500mg/kg)	7.20 ± 0.76***	7.33 ± 0.78***

3.5.3.2 Effect of selected *A. cordifolia* leaves extracts on serum marker enzyme levels of ethanol induced hepatotoxic rats

Table 3.15 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	79.88 ± 8.22**	196.28 ± 4.24**	210.46 ± 8.22**
5	AQEAC(500mg/kg)	68.28±6.76***	186.84±4.26***	198 ± 8.44***

3.5.3.3 Effect of *A. cordifolia* leaves extracts on biochemical parameters ethanol induced hepatotoxic rats

Table 3.16 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	0.68 ± 0.62**	8.28 ± 0.12**
5	AQEAC (500mg/kg)	0.56.0 ± 0.20***	6.18 ± 2.48***

3.5.4 Rifampicin induced hepatotoxicity

All of the animal groups went to sleep after receiving an intravenous dose of thiopentone sodium (40 mg/kg). Rats given RIF+INH slept for longer and later than their untreated counterparts, both measured in seconds (min). The sleep onset time and total sleep time of rats pretreatment with AEAC, AQEAC (500 mg/kg p.o.), and silymarin extracts were significantly earlier and shorter, respectively, compared to rats in the RIF+INH group.

Table 3.17 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	119.5 ± 4.50**	218.2 ± 4.08**
5	AQEAC (500mg/kg)	149.4 ± 5.22***	189.2 ± 4.96***

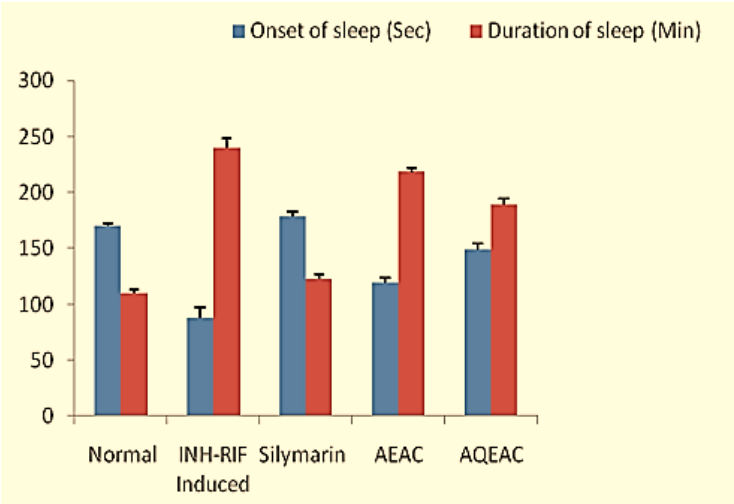


Figure 3.4 Effect of *A. cordifolia* leaves extracts on functional parameters in RIF+INH induced hepatotoxic rats.

3.5.4.1 Effect of *A. cordifolia* leaves extracts on physical parameters

Table 3.18 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	8.20 ± 2.42**	8.49 ± 2.28**
5	AQEAC (500mg/kg)	7.38 ± 0.24***	7.53 ± 0.24***

3.5.4.2 Effect of *A. cordifolia* leaves extracts on serum marker enzyme levels on RIF+INH induced hepatotoxic rats.

Table 3.19 Effect of *A. cordifolia* leaves 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***
4	AEAC (500mg/kg)	112.4 ± 7.20**	320.0 ± 6.93**	251.99 ± 9.31**
5	AQEAC(500mg/kg)	80.02±3.71***	259.0 ± 4.66***	230.15±6.30***

3.5.4.3 Effect of *A. cordifolia* leaves extracts on biochemical parameters RIF+INH induced hepatotoxic rats

Table 3.20 Effect of *A. cordifolia* leaves extracts on biochemical parameters rifampicin isoniazid induced hepatotoxic rats

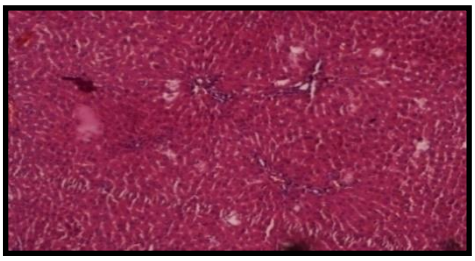
Sr.No.	Treatment/ Dose	Total Bilirubin (mg/dl)	Total Protein (gm/dl)
1	Normal	0.38 ± 0.06	9.57 ± 0.24
2	Induced (RIF+INH)	6.76 ± 8.04*	5.2 ± 0.16*
3	Standard (Silymarin)	0.42 ± 2.68***	9.60 ± 4.80***
4	AEAC (500mg/kg)	0.62 ± 2.62**	5.72 ± 8.01**
5	AQEAC(500mg/kg)	0.58.0 ± 4.23***	7.85 ± 4.02**

3.6 Histopathology

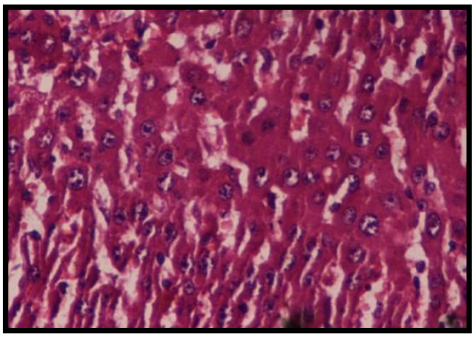
The hepatoprotective properties of A. cordifolia leaf extracts were the subject of biochemical studies that were corroborated by histopathological analyses. CCl4, paracetamol, ethanol, and

RIF+INH are all examples of hepatotoxicant; certain extracts may be utilised to treat histological abnormalities brought on by these substances. Figure following displays the outcome.

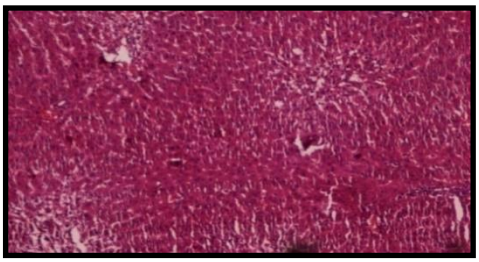
3.6.1 Effect of *A. cordifolia* leaves extracts on histopathological diagram of liver tissue in CCl4 induced hepatotoxic rats.



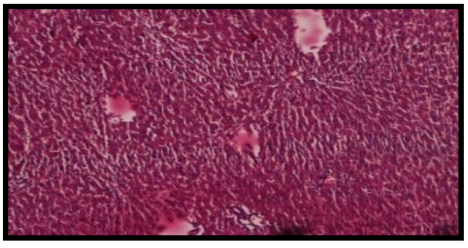
Normal: The design is a tried and true one. The portal triads, sinuses, and central veins are all normal. Hepatocytes are characterized by their moderate cytoplasm and their oval to round nuclei. There is no periportal inflammation.



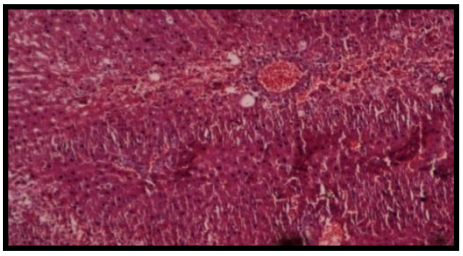
CCl_4 induced: There is less of the building now. It seems that there is a blockage in the sinusoids, portal triads, and central veins. There is very little cytoplasm in hepatocytes, and their nuclei are round or oval and have undergone a feathery degeneration. The area around the harbor is tense with fury.



Silymarin (25 mg/kg): There was no abnormality in portal area or hepatocyte size. Fibrous connective tissue enlargement and hepatotoxicity are hardly noticeable. Regeneration at its peak.



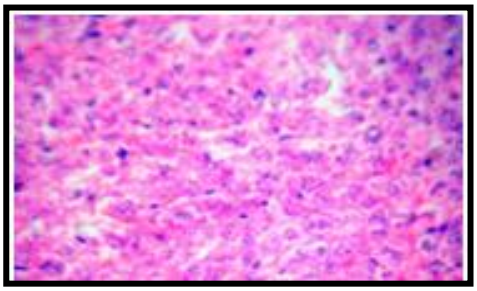
AEAC (500mg/kg): Liver with mild sign of hepatotoxicity, tissue with typical lobular arrangement. Minimal centrilobular necrosis. The portal triads show mild Peri portal inflammation composed of lymphocytes.



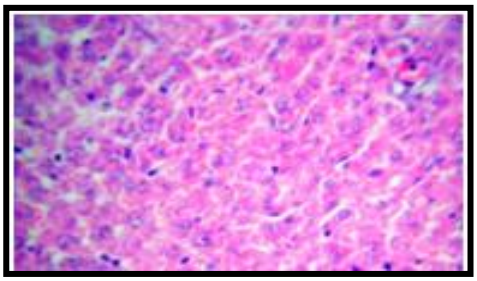
AQEAC (500mg/kg): Low levels of toxicity to the liver, which seems to be organized normally into lobes. Small amounts of necrosis in the centrilobular regions. Periportal inflammation in the portal triads is mild and is mediated by lymphocytes.

Figure 3.5 Effect of *A. cordifolia* leaves extracts on histopathological diagram of liver tissue in CCl4 induced hepatotoxic rats.

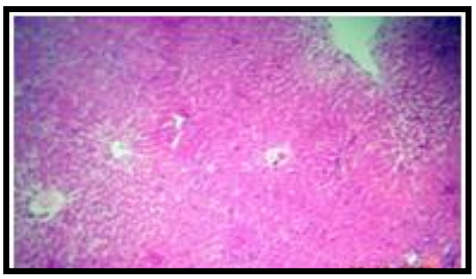
3.6.2 Effect of *A. cordifolia* leaves extracts on histopathological diagram of liver tissue in paracetamol induced hepatotoxic rats.



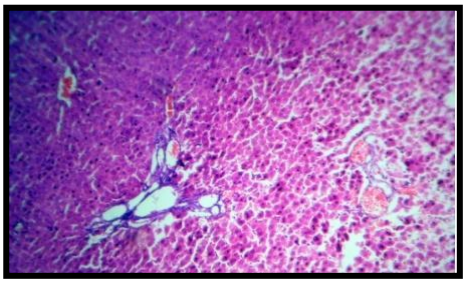
Normal: The design is a tried and true one. The portal triads, sinuses, and central veins are all normal. Hepatocytes' nuclei are oval to circular and they contain a negligible amount of cytoplasm. There is no periportal inflammation.



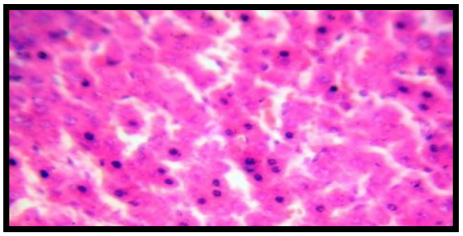
Paracetamol induced: The central veins are enlarged and swollen. Feathery senescence is a kind of cellular ageing that occurs in hepatocytes. Periportal inflammation in the portal triads is mild and is mediated by lymphocytes.



Silymarin (25 mg/kg): The major veins seem to be healthy. Feathering is a sign of ageing in hepatocytes. Periportal inflammation in the portal triads is mild and is mediated by lymphocytes.



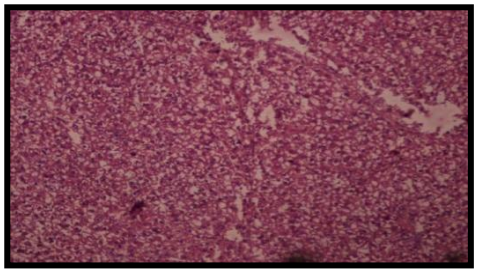
AEAC (500mg/kg): There is an increase in size, pleomorphic, and hyper chromaticity in hepatocyte nuclei. Periportal inflammation in the portal triads is mild and is mediated by lymphocytes. The central veins seem normal.



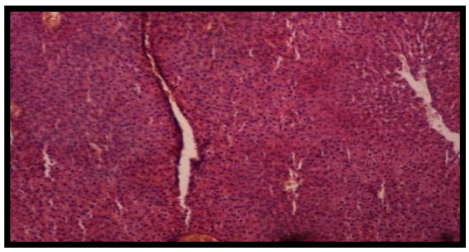
AQEAC (500mg/kg): This is a standard layout. Everything from the sinuses to the portal triads to the major veins seems healthy. Hepatocytes are characterized by their little cytoplasm and their round or oval nuclei.

Figure 3.6 Effect of *A. cordifolia* leaves extracts on histopathological diagram of liver tissue in paracetamol induced hepatotoxic rats.

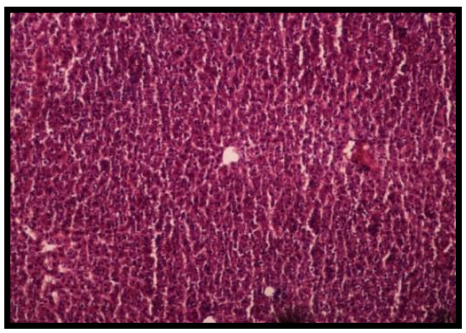
3.6.3 Effect of *A. cordifolia* leaves extracts on histopathological diagram of liver tissue in RIF+INH induced hepatotoxic rats.



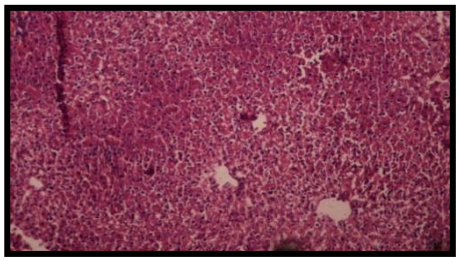
Normal: The design is a tried and true one. The portal triads, sinuses, and central veins are all normal. Hepatocytes are characterized by their moderate cytoplasm and their oval to round nuclei. There is no periportal inflammation.



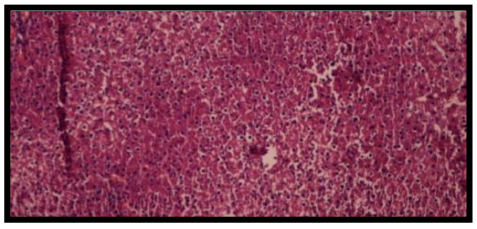
Rifampicin induced: Mild congestion and dilatation might be seen in the central veins. There have been shifts in hepatocyte steatosis and degeneration. Significant necrosis was seen in the portal tracts.



Silymarin (25mg/kg): Large, broad, and thick core veins are on display in the section. In general, hepatocytes are beneficial. It seems that there is a pattern to the triads in the portals. Fibrous connective tissue development is minimal, and liver damage is minimal. Maximum potential for healing.



AEAC (500mg/kg): Size and steatosis accumulation in hepatocytes varies. Inflammatory cells are present beside the parenchyma. While the degree of activity is low, hepatocellular necrosis is still present.



AQEAC (500mg/kg): There may be spots of necrosis in the structure. Hepatocytes are characterized by their round nuclei and little cytoplasm. It seems that there is a pattern to the triads in the portals.

Figure 3.7 Effect of *A. cordifolia* leaves extracts on histopathological diagram of liver tissue in RIF+INH induced hepatotoxic rats.

4 Conclusion

Coarsely powdered, shade-dried plant parts were soaked in a variety of solvent solutions to extract the hepatoprotective compounds. A preliminary physical and phytochemical test is performed on the concentrated extracts to determine the quality of the plant material and the presence of any active components. The powdered leaves and stem bark underwent a physicochemical study. The value of ash was determined in this research. Many phytoconstituents were detected in the samples during the phytochemical investigation. Glycosides, carbohydrates, proteins, amino acids, sterols, triterpenes, total phenolic compound, flavonoids, and saponin were some of the more notable ones. Liver damage in rats caused by CCl4, paracetamol, ethanol, and INH-RIF is treated with hepatoprotective therapy. Six mice were randomly assigned to receive either a control dose of AEAC (500 mg/kg) or extracts of AEAC, AQEAC, and silymarin (25 mg/kg, p.o.). In the groups that were subjected to hepatotoxicant, total bilirubin levels increased while total protein levels decreased significantly. Total bilirubin was significantly decreased and total protein was significantly raised after pretreatment with AEAC, AQEAC (500 mg/kg, p.o.), and silymarin (25 mg/kg). Serum enzyme marker proteins such SGPT, SGOT, ALP, and total bilirubin showed definite increases and decreases. The same holds true for liver illnesses in the context of clinical practice. For this reason, they serve as crucial markers for assessing liver health. The extracts utilised in this investigation significantly mitigated the increases in protein and the aforementioned enzymes. We may now conclude that the extracts have liver-protecting effects. Histopathological findings corroborated the effectiveness of AEAC, AQEAC, and silymarin in this investigation. Liver cytoarchitecture in animals treated with hepatotoxicant is drastically altered. The results for those with severe liver issues are consistent. Nevertheless, silymarin (25 mg/kg orally) and AQEAC (500 mg/kg)-treated AEAC mice had little liver alterations, and there was no alteration in hepatic cytoarchitecture. Hepatocyte regeneration was also seen, suggesting hepatoprotective effects. Improvements in serum marker enzyme levels, physical measures, functional parameters, and

histological tests were used to conclude that AEAC and AQEAC had a hepatoprotective effect. This lends credence to the ongoing scientific investigation of the same drugs used historically.

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