



International Journal of Current Trends in Pharmaceutical Research  
IJCTPR, 2013: Vol. 1(3): 194-204

## Evaluation of Protective Potential of *Nyctanthus Arbor Tristis* against Chloroform induced Hepatotoxicity in Rats

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### Abstract

*Nyctanthes arbor-tristis* Linn family Nyctantheaceae is widely distributed and easily available in the nature, therapeutic use of this plant is detailed in Charaka samhitha, Sushruta samhitha and other traditional systems of medicine. The seeds are cooling and astringent and are useful in vitiated condition of pitta. Seeds are useful in bilious disorders, yield fatty oil used as an illuminant. Oil from seed is used for poisonous bites, bowel complaints, epilepsy and for blackening the hair.<sup>1</sup> Bark has expectorant activity and has the power of removing pains and aches.<sup>2</sup> The drug is also used as Hepatoprotective.<sup>3</sup> The dried leaves was subjected to successive extraction using different solvent such as petroleum ether, chloroform, ethyl acetate, and methanol. These solvent extracts were subjected to a Phytochemical evaluation to detect the different chemical principle present i.e. Carbohydrates, Protein, Amino acids, Steroids, Glycosides, Alkaloids, Tannins, and Phenolic compounds and to carry out Chromatographic evaluation to isolate the chemical compounds. The present study was aimed at Chromatographic evaluation of *Nyctanthes arbor-tristis* Linn leaves. Methanol, Petroleum ether extract of leaves of *Nyctanthes arbor-tristis* Linn was screened in-vivo Hepatoprotective investigations. The Hepatoprotective activity of the various extracts of bark of *Nyctanthes arbor-tristis* Linn. was evaluated by the carbon tetra chloride method. In-vivo screening hepatoprotective shows significant activity in ethanolic extract of 100mg/kg and 200mg/kg dose when compared with standard hepatoprotective drug silymarin.

**Key words:** Chromatography, Hepatoprotective, Expectorant, investigation

### Introduction

Natural product is a source for bioactive compounds and has potential for developing some novel therapeutic agent. Over the last decade there has been a growing interest in drugs of plant origin and such drugs formed an important class for disease control. Herbs are staging a comeback and herbal 'renaissance' is happening all over the globe. The herbal products today symbolize safety in contrast to the synthetics that are regarded as unsafe to human and environment.<sup>4</sup> *Nyctanthes arbor-tristis* Linn belongs to family *Nyctantheaceae*. Commonly known as Harishringi. The vernacular names of the plant *Nyctanthes arbor-tristis* are in Hindi- Parijata, , Sanskrit- Harsinghar, tamil- Prajaktha, Marathi – Sephalika, Telugu- Night Jasmine.<sup>5</sup> It is distributed throughout India, in dry areas, mostly as follows in Arid and semi-arid regions of North-western India Upper Gangetic Plain and on the lower range of the Western Himalayas Occurring wild in the Sub-Himalayan region from Chenab to Nepal to 1500 m Chhota Nagpur, Rajasthan, Madhya Pradesh and Southwards to Godavari.<sup>6</sup>

The plant is a shrub or a small tree having 10 m height, young branches have angular stem. A hardy, hard shrub or a small tree, with grey or greenish white rough bark and quadrangular branches. It is essentially a warm season crop. A long period of warm and humid climate is required. It can be grown in mild climate, but is sensitive to forest. The optimum temperature requirement is 24-300 C for successful crop growth. It is mostly grown as mixed crop in kharif season.<sup>7</sup> one positive aspect of the use of medicinal plants is their low cost compared to the high prices of new synthetic drugs, which have become totally inaccessible to the vast majority of people. Another consideration in favors of the use of medicinal plants, when they are the only recourse available is that they have comparatively few side effects. Synthetic drugs, in general have very potent pharmacodynamic effects; but as they are active, many also have strong and possible dangerous and harmful side effects. Between 3 and 5 per cent of patient's hospital admission are attributed to side effects of Pharmaceuticals. On the contrary medicinal plants, with a few exceptions do not have great therapeutic potency, but neither do they have intense or serious side effects. Therefore, their direct

administration in folk medicine offers little risk. Thus, there exists a wide field for research in the phytochemistry of those hundred or more plants that are used in folk medicine in each country, research confirming the presence of pharmacodynamic chemicals such as alkaloids, glycosides to a lesser degree, and essential oils and other substances, indispensable the knowledge that justifies that practices of naturalist and folk medicine. The natural products have played the major role in drug discovery.<sup>8</sup>

### **Hepatoprotective Activity**<sup>9, 10, 11</sup>

Liver function tests are used to a suspected liver disease, to estimate progress and to assist in the differential diagnosis of jaundice. The biochemical manifestations of hepatotoxicity reflect the histological patterns of liver injury. Toxic necrosis leads to changes in the blood and to clinical features similar to those of acute viral hepatitis. Toxic steatosis leads to quantitatively similar but quantitatively more modest abnormalities. CCl<sub>4</sub>, paracetamol, halothane, methyl dopa etc. induced hepatocellular necrosis produces in blood high levels of enzymes released from the damaged liver. Most extensively studied in this regard, are the levels of the transaminase that may be increased to the values that are 10 to 200 folds the normal. Depressed levels of plasma coagulation factors are characteristic of hepatic necrosis, and in the usual clinical setting are reflected in the prolonged prothrombin time. Indeed the most useful clinical clues to severity of the necrosis are the prothrombin time and the bilirubin levels. The values of serum alkaline phosphatase and  $\gamma$ -GTP run parallel in liver damage. The levels of alkaline phosphatase, 5-nucleotidase, and leucine-aminopeptidase increase not more than 1-3 fold in response to necrosis. Albumin levels don't change appreciably in the early phase of acute necrosis, plasma cholesterol tends to be low or normal in acute hepatic necrosis. Microvascular steatosis resembling fatty liver of pregnancy, leads to less dramatic biochemical evidence of hepatic injury than does acute necrosis, values for serum transaminases (AST and ALT) in tetracycline toxicity increase from 5 to 20 folds the upper limit of normal. A series of tests are usually applied to screen the various functions of the liver, those of established value being:

#### **Examination of the urine for the presence of bilirubin and urobilinogen:**

The former is indicative of hepatocellular damage or of biliary obstruction. The presence of excessive urobilinogen can precede the onset of jaundice and forms a simple and sensitive test of minimal hepatocellular damage or of the presence of hemolysis.

#### **Estimation of total serum bilirubin:**

Normal value 5-17  $\mu$ mol/L.

#### **Estimation of serum enzyme concentration:**

Hepatocellular damage is accompanied by a raised level of a number of enzymes, in particular of  $\gamma$ -glutamyl transpeptidase, alanine aminotransferase (glutamic oxaloacetic transaminase) and by a moderately raised level of alkaline phosphatase.

#### **Serum transaminase (aminotransferase):**

Aspartate aminotransferase (AST) is a mitochondrial enzyme present in large quantities in heart, liver, skeletal muscle and kidney and the serum levels increase whenever these tissues are acutely injured, presumably due to release from damaged cells.

Alanine aminotransferase (ALT) is a cytosolic enzyme present in liver. The absolute amount is less than AST but a greater proportion is present there compared with heart and skeletal muscles, so that a serum increase is more specific for liver damage than AST. Transaminase determinations are useful in the early diagnosis of viral hepatitis. A high ratio of AST to ALT (Greater than two) may be useful in diagnosing alcoholic hepatitis and cirrhosis. Transaminase determinations are also useful in screening for liver injury due to drugs.

#### **Gamma glutamyl transpeptidase ( $\gamma$ -GTP):**

Gamma glutamyl transpeptidase ( $\gamma$ -GTP) is also known as cell surface or membrane bound enzyme. In human tissues,  $\gamma$ -GTP activity is prominent in kidney, pancreas, liver and prostate, the kidney showing the activity. In the liver, the enzyme has been demonstrated histochemically and biochemically in the luminal border of the epithelial cells lining the bile ductules slight histochemical activity has been observed with in the periportal hepatic cells and such activity may be increased in both inflammation and cirrhosis. The enzyme levels are increased in both cholestasis and hepatocellular disease.  $\gamma$ -GTP plays significant role in peptide nitrogen storage, in protein synthesis, in the regulation of tissue glutathione levels, and in amino acid transport across cell.

#### **Alkaline phosphatase:**

An increasing level of alkaline phosphatase is indicative of an obstruction lesion. The level rises in cholestasis and to a lesser extent, when liver cells are damaged. The increased serum alkaline phosphatase is of hepatic origin as the alkaline phosphatase of bile is derived from the liver. Raised levels are sometimes observed with primary and secondary tumors, even without jaundice or involvement of bone.

#### **5-Nucleotidase:**

The enzyme, whose function in the liver is not known, reflects hepatic and biliary tract abnormalities in a similar way to alkaline phosphatase.

**Leucine-amino peptidase:**

The activity of this enzyme in the serum has a similar usefulness to 5- nucleotides in that it is neither increased in children nor in patients with bone disease.

**Determination of plasma protein concentration an electro-phoretic pattern:**

Hepatocellular damage is accompanied by a fall in plasma albumin and a differential rise in the globulin fractions, in particular globulin. These changes form the basis of the flocculation tests of liver function.

**Bromsulphthalein Excretion test:**

This is a sensitive test of early cellular damage, and is of value in detecting its presence in the absence of jaundice.

**Immunological tests:**

Estimation of the levels of immunoglobulins and detection of autoantibodies is of value in the diagnosis of certain forms of chronic liver damage. The presence of hepatitis B surface antigen is indicative of serum hepatitis and the presence of alpha-fetoprotein suggests a hepatoma.

**Haemoglobin estimation:**

Red cell indices and report on blood films. Other tests used in the diagnosis of liver disease include scanning by means of ultrasound or radioisotope uptake, needle biopsy for histological examination and peritoneoscopy.

**Carbon Tetrachloride And Its Hepatotoxicity**

Transient exposure to toxic concentration of  $\text{CCl}_4$  vapour results in irritation of the eyes, nose and throat, nausea and vomiting, dizziness, headache. The most serious delayed toxic effect of  $\text{CCl}_4$  is hepatotoxicity.  $\text{CCl}_4$  inflicts wide-ranging effects on liver metabolism, including adverse reaction on DNA, RNA, protein synthesis, necrosis of hepatocytes and cirrhosis.  $\text{CCl}_4$  induced liver injury is initiated by formation of a reactive metabolite, trichloromethyl radical  $\text{CCl}_3$  by microsomal mixed function oxidase system (MFOS). This biotransformation is catalysed by a cytochrome P- 450 dependent monooxygenase. The activated  $\text{CCl}_3$  radicals binds covalently to the macromolecules and induces peroxidative degeneration of the biomembranes is one of the principle causes of  $\text{CCl}_4$  induced hepatotoxicity. Alcohol pretreatment markedly stimulates the toxicity of  $\text{CCl}_3$  due to accelerated production of reactive metabolites, because of the stimulation of microsomal drug metabolizing enzymes due to ethanol.

**Experimental Animals:**

Wistar albino rats (150-200 g) of both sexes were selected for the study. The rats were given food and water ad libitum. All the animals were kept under laboratory conditions for an acclimatization period of 7 days before carrying out the experiments. All studies were carried out in groups of 6 rats each. Each rat was housed separately in a metabolic cage. Experimental animals were obtained from the animal house of **Cadila Pharmaceuticals, Ahmedabad**. Pharmacological study was approved by **CPCSEA of Animal Ethical Committee of Cadila Pharmaceuticals, Ahmedabad**.

## Materials and Methods

**Collection and Authentication of plant**

The dried gummy bark of **Nyctanthes arbor-tristi** were collected in the day time locally from Anand District, Gujarat, India, in the month of September 2009. The plant was positively authenticated by Department of Botany, University of pune, India. A voucher specimen is deposited in the Department of Botany, University of Pune.

Equipments		
1.	Photoelectric colorimeter	Systronics 103, Ahmedabad
2.	Centrifuge Remi- T- 8A	Remi Scientific Indust, Bombay
3.	Micropipette	J. Mitra and Bros., Delhi
4.	Rotary microtoime	Yoroko Scientific Indust., Delhi
5.	Microscope(trioclar,with photomicrographicarrangement Olympus Vanox)	Olympus Optical Co., Japan
6.	PH meter	Control Dynamics, Bamgalore
7.	Electronic balance	Dhone, Instruments Pvt. Ltd. Calcutta
Diagnostic Kits		
1.	AST Kit	Span Diagnostics, Udhna, Surat
2.	ALT Kit	Span Diagnostics, Udhna, Surat
3.	Bilirubin Kit	Span Diagnostics, Udhna, Surat
4.	Total Protein Kit	Techno Pharmchem, Bahadurgarh.
Animals used		
1.	Albino rats	Obtained from Cadila Pharmaceuticals, Ahmedabad.
2.	Sex	Female/ Male

3.	Weight	120-150g
4.	Strain	Wistar
5.	Animal diet	Amrut, Rat Feed, Maharashtra
Drugs and Chemicals		
1.	Silymarin	Ranbaxy, Indore
2.	Carbon tetrachloride	E Merck, Mumbai
3.	NaCl	E Merck, Mumbai
4.	Diethyl Ether	CDH, Mumbai
5.	Formalin	Qualigens, Mumbai

**Experimental Procedure:**<sup>12,13,14,15</sup>

**Study protocol:** The rats were randomly divided into five groups, comprising of six animals in each group.

**Group - I**

Received 5% CMC 10ml/kg body weight once in a day orally for seven days. This group served as a normal control.

**Group - II**

Received equal mixture of  $CCl_4$  and olive oil (50% v/v) 3 ml/kg body weight intraperitoneally.

**Group - III**

Received ethanolic extract of bark of *Nyctanthes arbor-tristis* Linn 100 mg/kg orally once in a day for 7 days after  $CCl_4$  administration (3 ml/kg 50% v/v in olive oil)

**Group -IV**

Received ethanolic extract of bark of *Nyctanthes arbor-tristis* Linn 200 mg/kg orally once in a day for 7 days after  $CCl_4$  administration (3 ml/kg 50% v/v in olive oil)

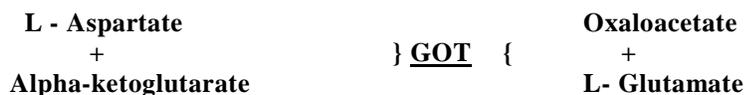
**Group -V**

Received Silymarin, the reference drug 25 mg/kg orally once in a day for 7 days after  $CCl_4$  administration (3 ml/kg 50% v/v in olive oil).

All the animals were sacrificed by cervical dislocation method on 8<sup>th</sup> day for the estimation of biochemical parameters and for histopathological studies. Blood was drawn from the carotid artery and serum was separated by centrifugation at 1000 rpm for the different assays.

**Serum Glutamate Oxaloacetate Transaminase (SGOT)**

The estimation based on the reaction, L-aspartate and alpha-ketoglutarate react in the presence of GOT in the sample to yield oxaloacetate and L-glutamate.



The oxaloacetate is reduced by malate dehydrogenase to yield L - malate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340nm.



The rate of reduction in absorbance is proportional to GOT activity in sample.

**1 SGOT (Enzymes/Coenzyme)****2 SGOT (Buffer)****Working reagent preparation:-**

Added 1.1 ml of 2 SGOT to one bottle of 1 SGOT and mixed well to dissolve and waited for 15 minutes prior to use. Use within 8 hrs.

**Specimen Collection: -**

Fresh clear serum under fasting condition with no haemolysis is the specimen of choice. Plasma collected with anticoagulants such as heparin or EDTA can also be used.

**Procedures:**

Type of reaction: Kinetic

Wavelength: 340nm

Flowcell Temp.: 37°C

Delay time: 60 seconds

Interval:30 seconds

No. of intervals:4

Sample volume: 50 microliters (0.05ml)

Working reagent volume: 1.0 ml

Factor: 3376

Light path : 1 cm

Zero setting with: Distilled water

Pipette into test tubes	Test
Working reagent (ml)	1.0
Sample (ml)	0.05

Mixed and after incubation at 37°C for 60 seconds, measured the absorbance at an interval of 30 seconds for 2 minutes at 340 nm.

### SGOT (U/L)

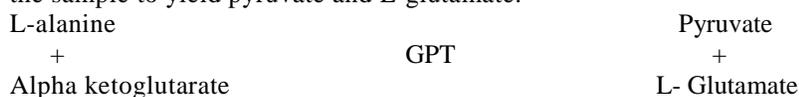
Group	I	II	III	IV	V
SGOT (U/L)	96.3±1.15	187.6±1.59	136.5±1.61**	104.9±2.32**	103.4±2.1**

Data are expressed as mean ± S.E, n=6

\* p < 0.01 Vs control \*\* p < 0.01 Vs Control.

### Serum Glutamate Pyruvate Transaminase (SGPT)

The estimation is based on the reaction of L-alanine with alpha ketoglutarate react in the presence of GPT in the sample to yield pyruvate and L-glutamate.



Pyruvate is reduced by lactate dehydrogenase to yield lactate with the oxidation of NADH to NAD. The reaction is monitored by measurement of the decrease in absorbance of NADH at 340 nm.



The rate of reduction in absorbance is proportional to GPT activity in sample.

#### 1 SGPT (Enzymes/Coenzyme)

#### 2 SGPT (Bufferd Substrate)

#### Working reagent preparation:-

Added 1.1 ml of 2 SGPT to one bottle of 1 SGPT and mixed well to dissolve and waited for 15 minutes prior to use. Used within 8 hrs.

#### Specimen Collection:-

Fresh clear serum under fasting condition with no haemolysis is the specimen of choice. Plasma collected with anticoagulants such as heparin or EDTA can also be used.

#### Procedures:

Type of reaction : Kinetic

Wavelength : 340nm

Flowcell Temp. : 37°C

Delay time : 60 seconds

Interval : 30 seconds

No. of intervals : 4

Sample volume : 50 microliters (0.05ml)

Factor : 3376

Light path : 1 cm

Zero setting with: Distilled water

<b>Pipette into test tubes</b>	<b>Test</b>
Working reagent (ml)	1.0
Sample (ml)	0.05

Mixed and after incubation at 37°C for 60 seconds, measured the absorbance at an interval of 30 seconds for 2 minutes at 340 nm.

**SGPT (U/L)**

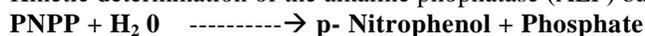
Group	I	II	III	IV	V
SGPT (U/L)	35.02±0.2	130.73±1.64	66.4± 1.73**	43.8± 1.21**	48.8±4.6**

Data are expressed as mean ± S.E, n=6

\* p < 0.01 Vs control \*\* p < 0.01 Vs Control.

**Alkaline Phosphatase (ALP)**

Kinetic determination of the alkaline phosphatase (ALP) based upon DGKC and SCE recommendations.



PNPP = Paranitrophenyl phosphate ALP = Alkaline

**Phosphatase****Reagents composition:****Reagent 1: (R1)**

Diethanolamine Buffer, pH 9.8 Magnesium Chloride

**Reagent 2: (R2)**

P-Nitrophenyl phosphate Working reagent

Mixed 4 volumes of Reagent 1 with 1 volume of Reagent 2 Stability: 4 weeks at 2-8°C

Sample:

Serum or Heparinised Plasma

Type of reaction : Kinetic

Wavelength : 405nm

Temperature : 37°C

Factor : 2750

Blank : Distilled water

Delay time : 60 seconds

No. of readings : 3

Time Interval : 60 seconds

Sample volume : 20 microliters (0.02ml)

Reagent Volume : 1000 µl

Laboratory procedure:

Sample - 20 µl

Working reagent - 1000 µl

Mixed and incubated for 1 minutes and measured the change of optical density per minute during 3 minutes.

**ALP (U/L)**

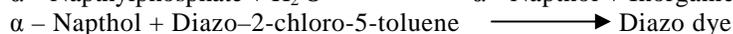
Group	I	II	III	IV	V
ALP(U/L)	15.89±0.70	97.5±6.9	84.7± 2.7*	35.8± 1.52**	33.1±2.3**

Data are expressed as mean ± S.E, n=6

p < 0.01 Vs control \*\* p < 0.01 Vs Control.

**Acid Phosphatase (ACP)**

Acid Phosphatase in acidic medium hydrolyses α - Naphthylphosphate into α - Naphthol and Phosphate. α - Naphthol reacts with diazo-2-chloro-5 - toluene forming an azo dye compound. The rate of formation of the azo compound at 405 nm is proportional to the total acid phosphatase activity. When the activity is measured in presence of tartrate, the prostatic activity is inhibited. The difference between total and non-prostatic activities corresponds to the prostatic fraction.

**ACP****Reagents:****Reagent 1 (Substrate):**

Citrate buffer, pH 5.30 80 mmol/L

α - Naphthylphosphate 3 mmol/L

Fast Red TR salt 1 mmol/L

**Reagent 2 (Tartrate):**

L- Tartrate 2 mmol/L

**Reagent 3 (Acetate Buffer):**

Acetate buffer, pH 5.005 mmol/L

Added 3ml distilled water into one bottle of reagent 1 and 5 ml distilled water into reagent 2 bottle. Mixed by gentle swirling till completely dissolved. Waited for 5 min. before using.

**General Systemic Parameters:**

Reaction type : Kinetic  
 Reaction slope : increasing  
 Wavelength : 405 nm  
 Flowcell temperature : 37°C  
 Delay time : 300 secs  
 No. of readings : 4  
 Interval : 60 secs  
 Sample Vol. : 100 µl  
 Reagent Vol. : 1 ml  
 Pathlength : 1 cm  
 Factor (Total) : 85  
 Factor (Non--prostatic) : 860  
 Zero setting with : Distilled water

**Set the instrument using above system parameters.**

Dispense into test tubes:

	Total Acid Phosphate
<b>Reagent 1</b>	1 ml
<b>Sample</b>	100 µl

Mixed and read immediately.

**ACP (U/L)**

Group	I	II	III	IV	V
ACP(U/L)	10.3±0.062	32.1±1.03	18.4± 0.41*	11.7± 0.12*	16.7±0.9*

Data are expressed as mean ± S.E, n=6

\* p &lt; 0.01 Vs control \*\* p &lt; 0.01 Vs Control.

**Bilirubin**

Systemic parameters for semi-auto analyzer:

Reaction type : End point with sample blank  
 Wavelength : 546 nm  
 Sample volume : 0.05 ml  
 Factor : 26.31  
 Delay : 1 sec  
 Number of readings : 1  
 Units : mg/dl  
 Blank : sample blank  
 Linearity : 25.0  
 Low Normal : 0.0  
 High Normal : 0.3 (Direct), 1.0 (Total)

**Procedure for semi-auto analyzer:-**

Pipetted out the reagents into test tubes labeled as below:

Reagent	Sample Blank Total SB <sub>T</sub>	Total Bilirubin T	Sample Blank Direct SB <sub>D</sub>	Direct Bilirubin D
Diazo A	0.25 ml	0.25 ml	0.25 ml	0.25 ml
Diazo B	---	0.025 ml	-----	0.025 ml
<b>Mix thoroughly, then proceed</b>				
Activator	0.25 ml	0.25 ml		

Distilled Water	0.5 ml	0.5 ml	0.75 ml	0.75 ml
Serum/Plasma	0.05 ml	0.05 ml	0.05 ml	0.05 ml

**For Direct Bilirubin :** Mixed well and read absorbance of SB<sub>D</sub> & D at 546 nm exactly after 1 minute at R.T.

**For Total Bilirubin:** Mixed well and read absorbance of SB<sub>T</sub> & T at 546 nm exactly after 5 minute at R.T.

#### Direct Bilirubin (mg/100ml of blood)

Group	I	II	III	IV	V
Direct bilirubin	0.24±0.02	0.50±0.08	0.48±0.04	0.30±0.018*	0.18±0.007*

Data are expressed as mean ± S.E, n=6

\* p < 0.01 Vs control \*\* p < 0.01 Vs Control.

#### Total Bilirubin (mg/100ml of blood)

Group	I	II	III	IV	V
Total bilirubin	0.38±0.04	0.89±0.02	0.71±0.06*	0.40±0.21*	0.21±0.01*

Data are expressed as mean ± S.E, n=6

\* p < 0.01 Vs control \*\* p < 0.01 Vs Control.

**Table 6.3 Effect of Ethanolic Bark Extract of *Nyctanthes Arbor-Tristis* Linn on CCL<sub>4</sub> Induced Hepatotoxicity In Rats**

Treatment	SGOT (U/L)	SGPT (U/L)	ALP (U/L)	ACP (U/L)	Bilirubin (mg/100 ml of blood)	
					Direct	Total
Normal	96.3±1.15	35.02±0.02	15.89±0.70	10.3±0.062	0.24±0.02	0.38±0.04
CCl <sub>4</sub> 1 ml/kg Intraperitoneally	187.6±1.59	130.73±1.64	97.5±6.9	32.1±1.03	0.50±0.08	0.89±0.02
Plant extract (100 mg/kg orally)	136.5±1.61**	66.4±1.73**	84.7±2.7*	18.4±0.41*	0.48±0.04	0.71±0.06*
Plant extract (200 mg/kg orally)	104.9±2.32**	43.8±1.21**	35.8±1.52**	11.7±0.12*	0.30±0.018*	0.40±0.21*
Silymarin (25 mg/kg orally)	103.4±2.1**	48.8±4.6**	33.1±2.3**	16.7±0.9*	0.18±0.007*	0.21±0.01*

Data are expressed as mean ± S.E, n=6

\* p < 0.01 Vs control \*\* p < 0.01 Vs Control by students 't' test

#### Histopathological Studies of the liver:

Immediately after sacrifice of rat, the liver was dissected out, washed with ice-cold saline, The liver sections were taken from each lobe of the liver and fixed in 10 % neutral formalin solution and embedded in paraffin by employing the standard technique, 5µ in thick sections were cut and stained with hematoxylin-eosin for histopathological examination. The remaining liver was cut into approximately 50 - 100 mg portions on ice and stored separately at 7°C in plastic vials. The sections were taken by using microtome. Fig 1

**Group -I**

The control group animals have shown normal architecture.

**Group --II**

CCl<sub>4</sub> challenged animals have shown congested blood vessel, necrosis of hepatocytes, portal infiltration, fatty change, kupffer cell hyperplasia and hydropic changes.

**Group -III**

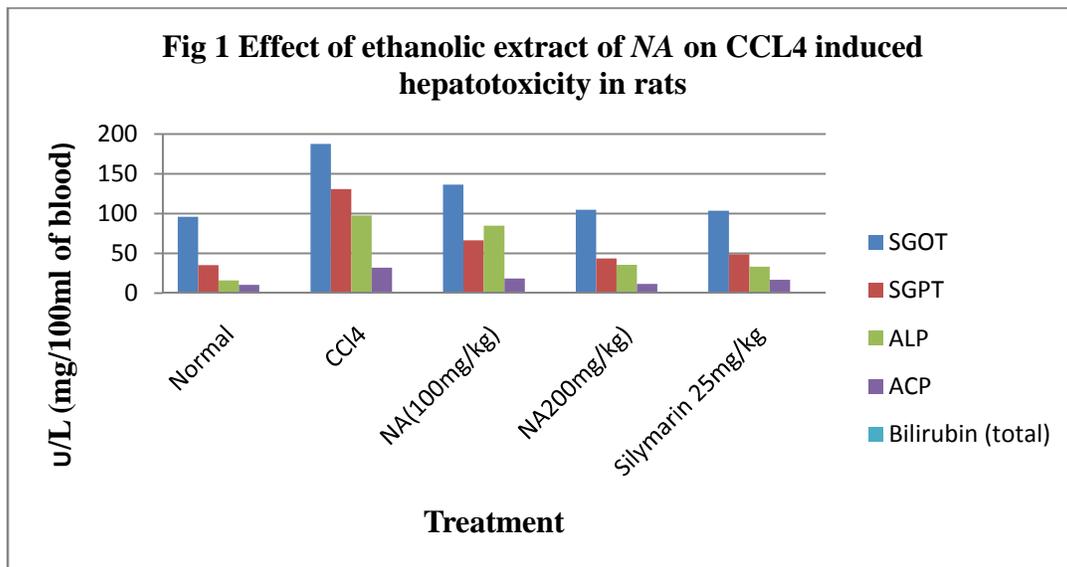
The animals treated with 100mg/kg of the ethanolic bark extract of *Nyctanthes arbor-tristis* Linn. have shown the necrosis which is more severe form of injury is somewhat recovered. It has also shown fatty change.

**Group -IV**

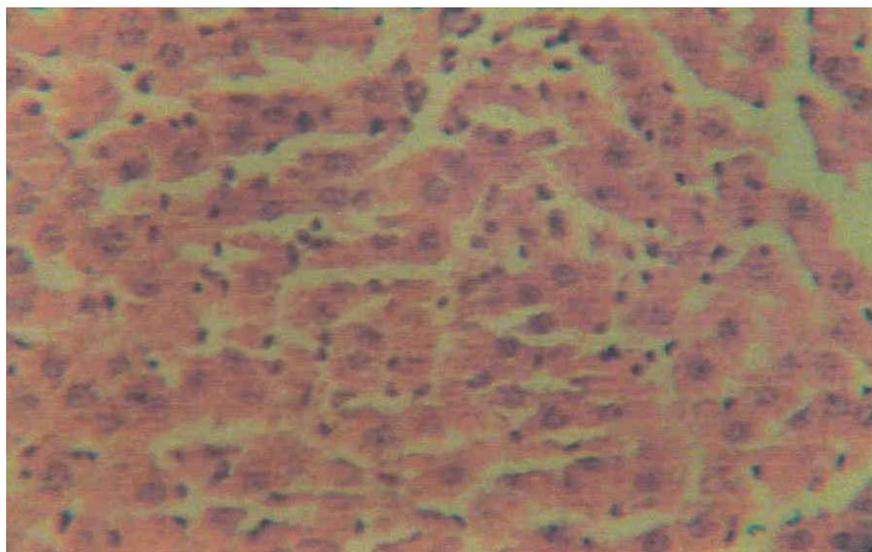
The animals treated with 200 mg/kg of the ethanolic bark extract of *Nyctanthes arbor-tristis* Linn, have shown the necrosis which is markedly prevented. Milder form of injury like fatty change and reduced necrosis persisted by the extract.

**Group -V**

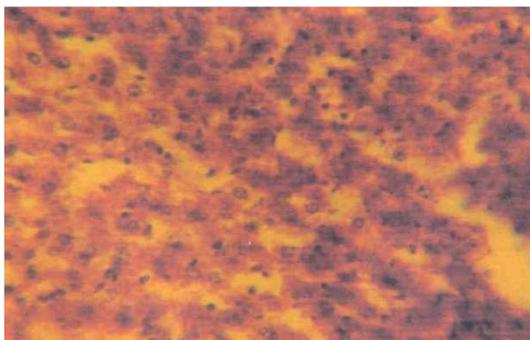
The animals treated with Silymarin have shown ballooning degeneration and fatty changes of hepatocyte necrosis which is more severe form of injury is markedly prevented.



**Fig 1 Histopathological Studies of Rat Liver**

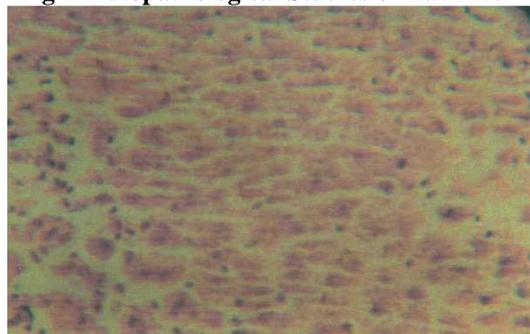


**Group – I (Normal Control)**

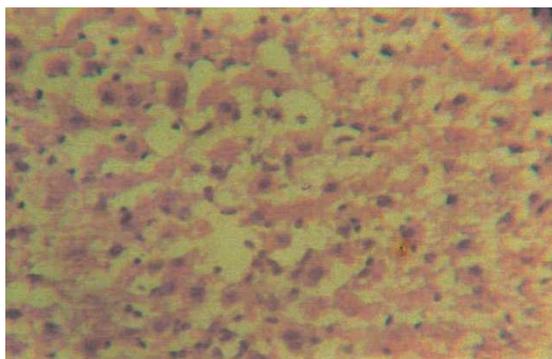


**Group – II CCl<sub>4</sub> Challenged**

**Fig 2 Histopathological Studies of Rat Liver**

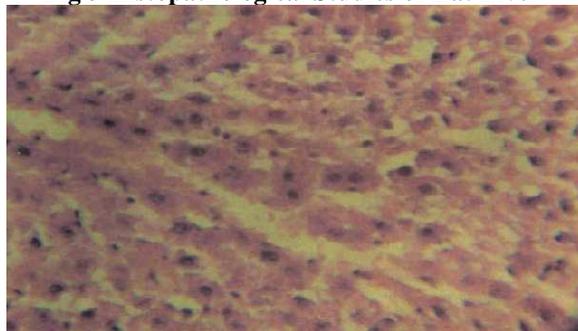


**Group – III (100mg/kg ethanolic bark extract)**



**Group – IV (200mg/kg ethanolic bark extract)**

**Fig 3 Histopathological Studies of Rat Liver**



**Group – V 25 mg/Kg Silymarin (standard reference)**

**Procedure :**

Five Wistar Albino rats of either sex having weight 180-230 gm were used for the study. The animals were obtained from the animal house of Cadila pharmaceuticals, Ahmedabad. Fixed dose levels of 50, 100, 200, 500, 1000 were given initially to allow identification of a dose producing evident toxicity for the ethanolic bark extract of *Nyctanthes arbor - tristis* Linn. After giving the dose the toxic signs were observed within 48 hours, Food was

withheld for a further period of 3-4 hours after administration of drug. The further 2000 mg/Kg was administered after the last dose and observed for the mortality. As most of the crude extracts possess LD more than 2000 mg/Kg ( $LD_{50} > 2000$  mg/kg). Body weight of the rats before and after administration was noted and any changes in skin, fur, eyes, mucous membranes and also respiration and behavior pattern were observed. Also signs of tremors, convulsions, salivation, diarrhoea, sleep and coma were noted. The onset of toxicity and signs of toxicity were also noted, if any.

### Observation and Results

There was no any considerable changes in body weight before and after administration and signs of toxicity were not present. Thus the  $LD_{50}$  of the ethanolic bark extract as per OECD guidelines - 420 is greater than 2000mg / Kg ( $LD_{50} > 2000$  mg/kg)

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