

PROTECTIVE ROLE, *IN-VITRO* FREE RADICAL SCAVENGING ACTIVITIES OF ALANGIUM SALVIFOLIUM (*LINN.*) AGAINST CCL₄ INDUCED HEPATIC DAMAGE IN RATS

PABBA PARAMESHWAR^{1*}, YELLU NARASIMHA REDDY²

¹Jyothismathi institute of Pharmaceutical sciences, Karimnagar, ²Department of Pharmacology, University College of Pharmaceutical Sciences, Kakatiya University, Warangal, Andhra Pradesh, India. Email: param_pabba@yahoo.com

Received: 14 Jun 2013, Revised and Accepted: 02 Feb 2014

ABSTRACT

Many hepatoprotective herbal preparations have been recommended in alternative system of medicine for the treatment of hepatic disorders. There are few or no systemic studies have been done on protective efficacy of *Alangium salvifolium* (Alangiaceae) to treat liver diseases. Hepatoprotective and antioxidant activity of ethanolic extracts was evaluated against CCl₄-induced liver damage in rats. Liver damage was evidenced by elevated levels of biochemical parameters such as serum glutamate oxaloacetic acid transaminase, glutamate pyruvic transaminase and serum alkaline phosphatase. Treatment with ethanolic extracts of *Alangium salvifolium* (300,500mg/kg, p.o.) produced a significant reversal in the above biochemical parameters, and reducing power, superoxide anion scavenging activity and reduced histopathological scores. These findings suggest that the extracts of *Alangium salvifolium* possess significant hepatoprotective and antioxidant properties.

Keywords: Hepatoprotective activity, Antioxidant activity, *Alangium salvifolium*, *Silymarin*, CCl₄

INTRODUCTION

Liver is the one of the largest organ in the body and chief site for metabolic events in the body so it as unique role in the maintenance, regulation of homeostasis of the body it has interrelated with all the biochemical pathways to growth, fight against disease. The major function of the liver is carbohydrate, protein and fat metabolism, detoxification, secretion of bile and storage of vitamin. Thus, to maintain a healthy liver is a crucial factor for overall health and well being. But it is continuously and variedly exposed to environmental toxins like CCl₄ alcohol and prescribed over the counter drug which can eventually lead to various liver ailments like hepatitis, cirrhosis and alcoholic liver disease.

Thus liver disease are some of the fatal thy disease in the world today. They pose a serious challenge to public health. Modern medicines have little to offer for elevation of hepatic disease and it is chiefly the plant based preparations which are employed for their treatment of liver disorders.

But there are not much drugs available for the treatment of liver disorders there fore many folk remedies from plant origin are tested for its potential hepatoprotective and free radical scavenging activity of liver damage in experimental animals models CCl₄ induced hepatotoxicity model is selected for study of hepatoprotective and free radical scavenging activity of plant extract *Alangium salvifolium* (L.f) Wang belongs to famil Alangiaceae.(Chatterjee A, et al, 1995.)

Locally it called as Ankolam. Alangiaceae is a monogeneric family of trees and shrubs found in tropical and subtropical region. There are nearly twenty one species of *Alangium* grouped into four sections *Alangium*, *Conostigma*, *Marlea* and *Rhytidendra*. Other two different varieties of this drug namely *Angolam* and *Karaangolam*. They correspond to *Alangium salvifolium*, subspecies *salvifolium* and *hexapetalum* respectively. The plant is distributed in dry regions, plains and lower hills in India, Africa, Srilanka, Indochina and China. Root is used in diarrhea, paralysis, piles and vomiting.

They are acrid, astringent, emollient, anthelmintic, thermogenic, diuretic and purgative. Root is useful for external application in acute case of rheumatism, leprosy and inflammation and internal application in cases of bites of rabbit and dogs. Antibacterial compound was isolated from the flower of *Alangium salvifolium*. Recent phytochemical studies of this plant resulted in the isolation of several flavonoids and phenolic compound (Kirtikar KR et al).

MATERIALS AND METHODS

Chemicals

Silymarin was used as a standerd hepatoprotective agent and was obtained as a gift sample from Micro Labs., Ltd., Hsour, Bangalore, india; Methanol from S S Pharma distributors Warangal, Tolune, Ethyl acetate, butanon from Merck Specialites Mumbai, CCl₄, formaldehyde from S.D fine Chemicals, Mumbai, olive oil from local Ayurvedic stores, Thiopental sodium (thiosol)- Neon Labs., Mumbai; and normal Phase pre- coated Chromatographic Plates – Merck, Germany.

Alanine transaminase (ALT), Aspartate transaminase (AST), Alkaline phosphate (ALP), Total Bilirubin (TBL), Total protein (TPL) and Albumin (ALB) by manual methods were purchased from Span Diagnostic Ltd., surat, india. The biochemical analytical kits for auto analyzer were purchased from Merck specialties Private Ltd, Mumbai india. All other chemical and solvents used were of analytical grade

Animals

Male albino rats, weighing about 150–200 g obtained from the Mahaveer Enterprizes, Bagh Ambarpet, Hyderabad (CPCSEA registration no: 146/1999/cpcsea) and the animals were kept in the animal house of Jyothismathi institute of Pharmaceutical Sciences, Timmapur, Karimnagar, A.P. at room temperature of 25 - 30°C and at 45 - 55% relative humidity for 12 hr, each of dark and light cycle. The animals were feed with rat pellets (Hindustan Lever Limited, Bangalore, India) and filtered water.

Animal studies in the work have been strictly performed as per the Institutional Animal Ethical Committee (IAEC) constituted under the guidelines of Committee for the Purpose of Control and Supervision on experimental Animal (CPCSEA), Ministry of Environment, and Govt. of India.

Collections of plant materials

The leaves of *Alangium salvifolium* (ASF) collected from Shathavahana university campus, Karimnagar Andhra Pradesh India between November and December; the plant was authenticated by the Professor R. Odaiah, SSR Govt. Degree & PG College Karimnagar Andhra Pradesh, India. A voucher specimen (SSR 2012/1/19) has been preserved in our laboratory. The plants were washed thoroughly in tap water, shade dried and powdered.

Determination of acute toxicity

Acute toxicity study was conducted for ethanolic extract of ASF by stair case method following OECD guidelines (K. Dash et al). There

was no lethality up to a dose of 1000 mg/kg, p.o. Nearly one tenth of the maximum dose of the extract that is 300, 500 mg/kg (p.o) was selected as the plant extract dose in all experiments

Hepatoprotective activity

In the present study, the animals were pretreated with test extract/ fractions before inducing liver damage with CCl_4 . seven days after acclimatization, the rats were divided into nine groups (I-VI), each group consisting of six animals. All animals were kept on same diet for 7 days. Group - I served as a control and received 1ml/kg of 2%w/v gum acacia in distilled water p.o. for seven days. Group - II treated with vehicle (1 ml/kg of 2%w/v gum acacia in distilled water p.o.) daily for 15 days followed by CCl_4 on the seventh day. Group - III (standatrd silymarin) animals were administered with 50 mg/kg of silymarin p.o. for seven days followed by CCl_4 administration p.o. Group - IV-VI test groups were treated in the similar way using methanolic, petroleum ether and water extracts of different doses respectively followed by CCl_4 administered p.o on the seventh day (Brijesh.K.,Tiwari., et al.,2009).

All the rats were anaesthetized with thiopental sodium (60 mg/kg intraperitoneally), 36 hrs after administration of CCl_4 , blood was collected from common carotid by carefully opening the neck region of the rats. After blood collection, the blood samples were allowed to coagulate at room temperature for at least one hour. Serum was separated by centrifugation at 3000 rpm for 30 minutes and then analyzed for TB, ALT, AST, ALP, TP and ALB levels. The animal were dissected, the livers were carefully removed, washed with 0.9% saline solution and preserved in formalin solution (10% formaldehyde) for histopathological studies.

Percentage protection = $1 - T - V / C - V \times 100$

Where "T" is the mean value of the drug and CCl_4 "C" is the mean value of CCl_4 alone and V is the mean value of vehicle treated animals (Brijesh.K.,Tiwari., et al.,2009).

Estimation of biochemical parameters

The following are the biochemical parameters estimated to evaluate the effect of the test materials against the experimentally induced hepatotoxicity caused by different agents:

Alanine amino transferase (ALT) Aspartate amino transferase (AST) Serum alkaline phosphatase (ALP), Total protein levels (TP) Total serum bilirubin (TB) Albumin levels (ALB) Glutathione levels (GSH), Malondialdehyde levels (MDA)

Assessment of in vitro free radical Scavenging activity

Inhibition of DPPH radical

The free radical scavenging activity of the extract was analyzed by the DPPH (1, 1-diphenyl-2-picryl hydrazyl) assay. A total of 2ml of the test extract, at concentrations ranging from 1 ug/ml to 100 ug/ml each, was mixed with 1ml of 0.5Mm DPPH (in methanol).the absorbance at 517nm was taken after 30 min of incubation in the

dark room temperature. The experiment was don in triplicate.the percentage antioxidant activity was calculated as follows:

% antioxidant activity [AA] = $100 - \frac{[Abs_{sample} - Abs_{blank}] * 100}{abs \text{ ml of methanol plus } 2.0 \text{ ml of the extract was used as the blank while } 1.0 \text{ ml of the } 0.3Mm}$

DPPH solution plus 2.0 ml of methanol was used as the negative control. Ascorbic acid was used as the reference standard. (Shah P.A, et al., 2009)

Determination of lipid peroxidation:

Lipid peroxidation was estimated by thiobarbituric acid (TBA) reaction with malondialdehyde (MDA). To 1 mL of supernatant, 0.5 mL of 30% trichloroacetic acid (TCA) was added followed by, 0.5 mL of 0.8% TBA. The tubes were kept in a shaking water bath for 30 min at 80 °C. After 30 min of incubation the tubes were taken out and kept in ice-cold water for 10 min. These were then centrifuged at 800 g for 15 min. The amount of MDA was assessed by measuring the absorbance of supernatant at 540 nm at room temperature against an appropriate blank. The percentage inhibition of Lipid peroxidation was calculated using the equation:

% inhibition = $A_0 - A_1 / A_0 \times 100$

Where, A0 is the absorbance of the control without extract and A1 is the absorbance of the sample extract (Gupta M, Mazumdar UK, et al) (Amimoto, T et al., 1995).

Statistical analysis

Results were expressed as mean \pm SEM. Statistical analysis was performed with one way analysis of variance (ANOVA) followed by student's t-test. P values less than 0.05 was considered to be statistically significance when compared with the control.

RESULTS

Hepatoprotective activity of ethanol extract of Alangium selvifolium leaves

The results of this study are presented in Table. 1.

Biochemical parameters: The elevated serum AST, ALT, ALP and TB levels were significantly ($P < 0.001$) reduced by the standard. The test groups ASEE-300 and ASEE-500mg/kg.b.w also exhibit a significant protective effect on the serum levels and also increase the reduced serum TP and ALB levels. The ASEE-500 showed a better hepatoprotective activity ($P < 0.001$) than ASEE-300. The high percentage protection was observed with ASME-500 was also comparable to the reference standard drug Silymarin with to all the parameters.

Hepatoprotective activity of Petroleum ether extract of Alangium selvifolium leaves:

The results of this study are presented in Table. 2

Table 1: The results of this study are presented

Groups	ALT(IU/L)	AST(IU/L)	ALP(KA/dL)	TB(mg/dL)	TP(gm%)	ALB(gm%)
Control	55.84 \pm 4.51	43.22 \pm 0.11	36.22 \pm 0.57	0.84 \pm 0.30	4.74 \pm 0.06	2.49 \pm 0.11
Toxic	110.00 \pm 3.25	95.20 \pm 4.01	95.01 \pm 3.00	1.62 \pm 0.11	0.77 \pm 0.17	0.62 \pm 0.09
Standard (Silymarin-50)	57.23 \pm 4.18	59.19 \pm 2.50	39.56 \pm 2.18	0.87 \pm 0.18	4.29 \pm 0.16	3.96 \pm 0.15
ASEE-300	58.01 \pm 2.12	42.35 \pm 2.88	40.51 \pm 4.25	0.91 \pm 0.19	3.28 \pm 0.13	2.09 \pm 0.52
ASEE-500	48.21 \pm 3.15	39.35 \pm 3.08	36.27 \pm 2.89	0.76 \pm 0.19	4.68 \pm 0.14	3.57 \pm 0.47

Biochemical parameters

the elevated serum AST, ALT, ALP and TB levels were significantly ($P < 0.001$) reduced by the standard. The test groups ASPEE-300 and ASPEE-500mg/kg.b.w also exhibit a significant protective effect on the serum levels and also increase the reduced serum TP and ALB levels. The ASPEE-500 showed a better hepatoprotective activity ($P < 0.001$) than ASPEE-300. The

highpercentage protection was observed with ASPEE-500 was also comparable to the reference standard drug Silymarin with to all the parameters.

Hepatoprotective activity of Water extract of Alangium selvifolium leaves

The results of this study are presented in Table.3.

Biochemical parameters

the elevated serum AST, ALT, ALP and TB levels were significantly ($P < 0.001$) reduced by the standard. The test groups ASWE-300 and ASWE-500mg/kg.b.w also exhibit a significant protective effect on the serum levels and also increase the reduced serum TP and ALB

levels. The ASWE-500 showed a better hepatoprotective activity ($P < 0.001$) than ASWE-300.

The high percentage protection was observed with ASWE-500 was also comparable to the reference standard drug Silymarin with to all the parameters.

Table 2: The results of this study are presented

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA/dL)	TB (mg/dL)	TP (gm%)	ALB (gm%)
Control	55.84±4.51	43.22 ± 0.11	36.22±0.57	0.84±0.30	4.74±0.06	2.49±0.11
Toxic	110.00±3.25	95.20 ± 4.01	95.01± 3.00	1.62±0.11	0.77±0.17	0.62±0.09
Standard (Silymarin-50)	57.23±4.18	59.19 ± 2.50	39.56±2.18	0.87±0.18	4.29±0.17	3.96±0.15
ASPEE-300	67.29±2.01	64.23±1.09	61.65±3.28	1.31±0.19	3.89±0.47	2.28±0.46
ASPEE-500	53.20±2.36	48.36±2.14	51.05±1.95	0.95±0.14	4.29±0.25	3.79±0.37

Table 3: The results of this study are presented

Groups	ALT (IU/L)	AST (IU/L)	ALP (KA/dL)	TB (mg/dL)	TP (gm%)	ALB (gm%)
Control	55.84±4.51	43.22 ± 0.11	36.22±0.57	0.84±0.30	4.74±0.06	2.49±0.11
Toxic	110.00±3.25	95.20 ± 4.01	95.01± 3.00	1.62±0.11	0.77±0.17	0.62±0.09
Standard (Silymarin-50)	57.23±4.18	59.19 ± 2.50	39.56±2.18	0.87±0.18	4.29±0.17	3.96±0.15
ASWE-300	60.36±1.98	68.65±1.96	58.29±2.98	1.48±0.20	2.89±0.33	2.00±0.37
ASWE-500	55.21±3.21	46.98±3.19	44.28±2.19	0.65±0.11	4.81±0.19	3.97±0.28

Groups	Concentration Mcg/ml	% Inhibition	IC ₅₀ values Mcg/ml
Ascorbic acid	5	33.25	16.51
	10	40.26	
	15	49.30	
	20	54.56	
	25	61.85	
	30	75.25	
EAF-ASEE	100	14.70	639.54
	200	25.85	
	400	37.71	
	600	47.62	
	800	59.58	
	1000	86.14	

Groups	Concentration Mcg/ml	% Inhibition	IC ₅₀ values Mcg/ml
Ascorbic acid	50	26.87	228.02
	100	41.50	
	400	66.50	
	600	90.63	
EAF-ASEE	100	19.31	504.63
	200	25.48	
	400	40.49	
	600	58.39	
	800	61.66	

The histopathological studies: (Fig. 1.)

the histopathological; study indicated that the hepatic damage induced by CCl₄ were remarkably reduced by the standard Silymarin, test showed a reduced fatty changes, necrosis and broad infiltration of lymphocyte produced by CCl₄. the effect with test extract almost comparable to the standard group

Anti-oxidant activity

Table. 1. Shows DPPH Radical scavenging activity of the ethanolic, petroleum ether and water extracts of the Alangium salvifolium was compared with those of standard ascorbic acid. The DPPH radical scavenging abilities of the extracts (86.14%) were found to be comparable than those of standard Ascorbic acid (75.25%).

Table 2. Shows the dose response results of nitric oxide scavenging and super oxide anion scavenging of the ethanol extracts of leaves of

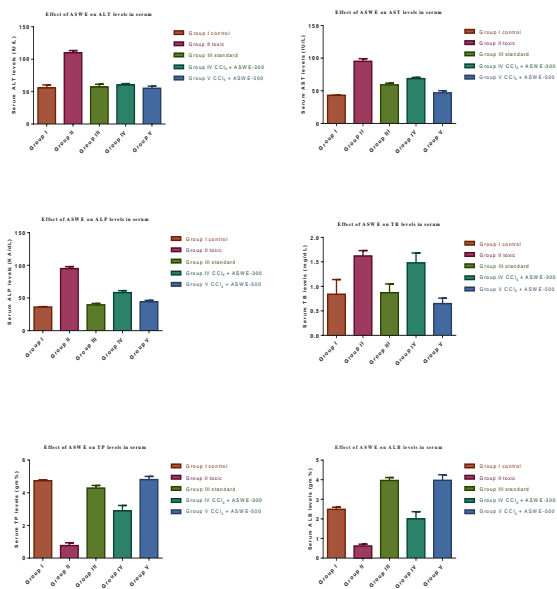
Alangium salvifolium. The extract reduced the generation of nitric oxide radical from sodium nitroprusside solution. This showed marked nitric oxide scavenging of the extract (90.63%).also the extract showed significant superoxide scavenging activity (61.63%)

DISCUSSION

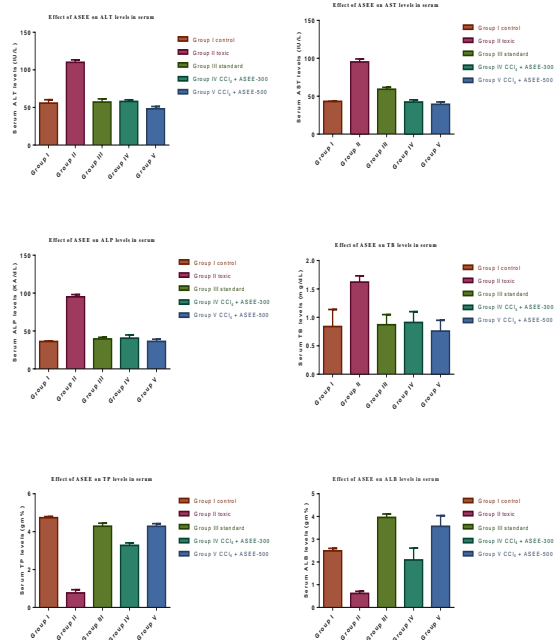
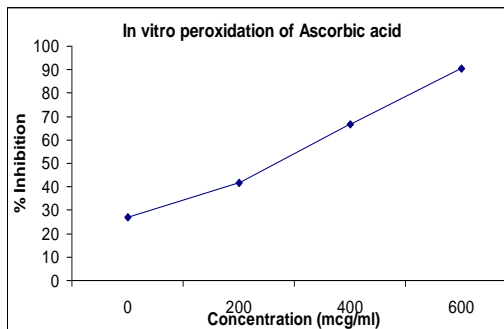
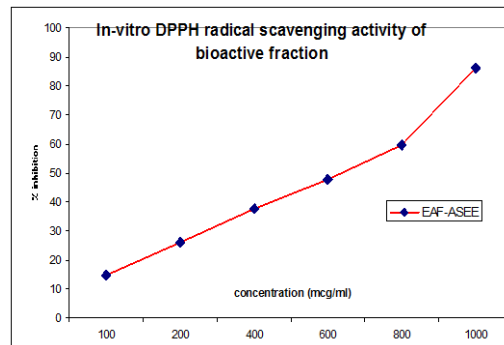
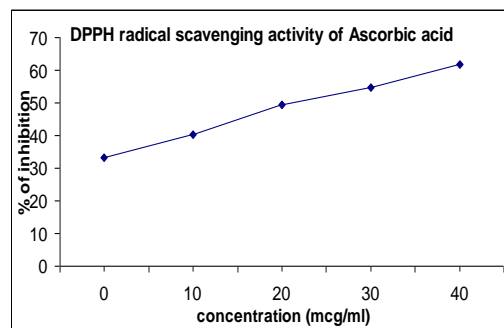
CCl₄ is commonly used for induction of experimental liver toxicity. This toxic chemical causes peroxidative degradation of the adipose tissue, resulting in fatty infiltration of the hepatocytes. Its metabolites such as trichloromethyl radical (.CCl₃) and trichloromethyl peroxy radical (CCl₃O₂) are involved in the pathogenesis. As shown in fig 2, CCl₄ causes changes around the central vein in the liver and other oxidative damages with the leakage of marker enzymes like ALT, AST and ALP in the serum. Treatment with ethanolic, petroleum ether and water extracts significantly reduced the elevated levels of the enzymes

towards the respective normal value that is indication of stabilization of plasma membrane as well as repair of hepatic tissue damage caused by CCl4

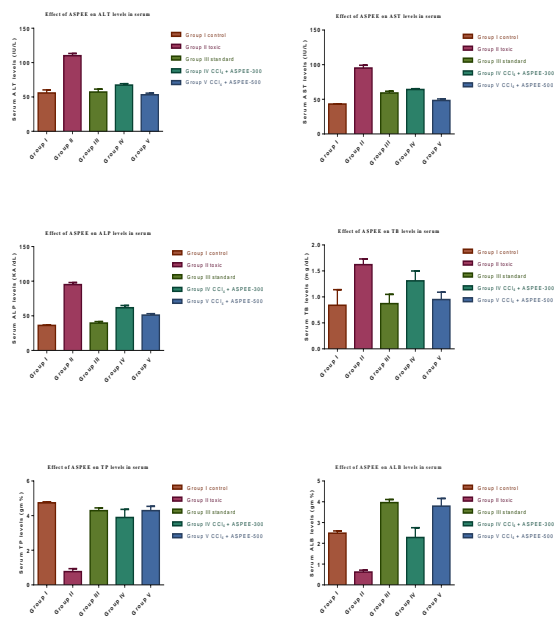
The qualitative phytochemical investigations on extracts Alangium salvifolium also showed positive for flavonoids by ferric chloride, alkaline reagent, furthermore, flavonoids constituents of plant possess antioxidant properties. Administration of ethanolic extract of Alangium salvifolium showed significant anti oxidant activity.



Hepatoprotective activity of Water extract of Alangium selvifolium leaves



Hepatoprotective activity of ethanol extract of Alangium selvifolium leaves



Hepatoprotective activity of Petroleum ether extract of Alangium selvifolium leaves:

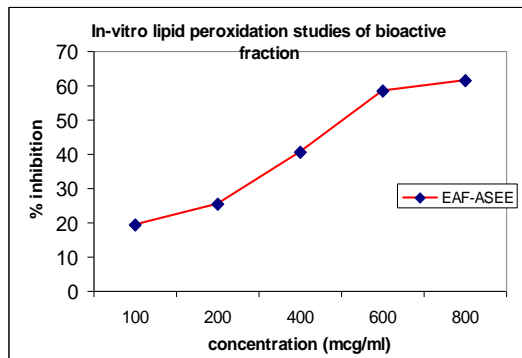
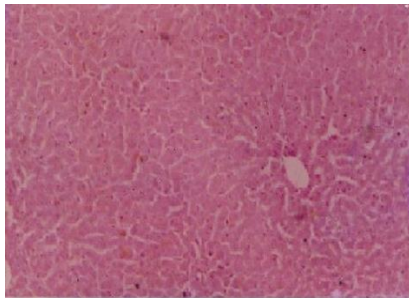
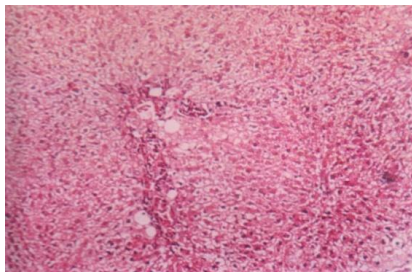


Fig. 1: Histological sections of rat liver obtained from different treatment groups.

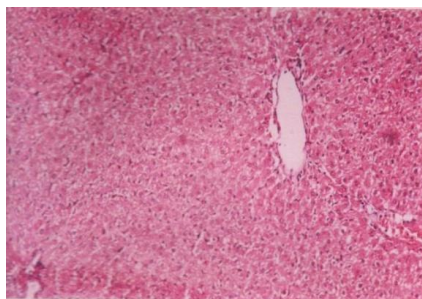
(A) Normal control



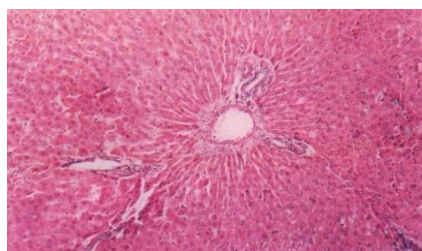
(B) CCl₄ control



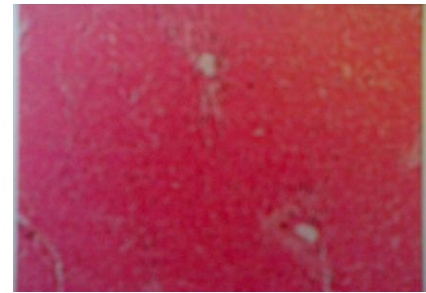
(C) Reference drug



(D) Alcoholic extracts 300 mg/kg



(E) Alcoholic extract 500mg/kg



ACKNOWLEDGEMENT

The authors thank to the management of Jyothishmathi Institute of Pharmaceutical sciences and Kakatiya University for providing necessary facilities.

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