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Research Article

EVALUTAION OF *PHYLANTHUS AMARUS* SEEDS EXTRACT FOR HEPATOPROTECTIVE ACTIVITY IN WISTAR RATS

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ABSTRACT

Hepatic systems is highly prone to attack by oxidative stress and generation of excessive concentration of free radicals. This results in tissue necrosis and damage to the liver. To search for effective and safe treatment of liver disease we have made an attempt to find out the hepatoprotective activity on *Phyllanthus amarus* seeds. The 70% methanolic extract of *Phyllanthus amarus* seeds (150mg/kg, 250 mg/kg and aqueous extract of 300 mg/kg) were used for in-vivo studies to analyze the reparative activity of liver damage due to CCl4, Paracetamol and thioacetamide induced hepatotoxicity in Wister rats. Liver functions were assessed by collecting the blood samples from each group & evaluating the biochemical parameters. Histopathological studies were done by isolating the liver of all the groups.

Treatment with 70% methanolic extract has brought back the elevated levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), bilirubin, protein, serum cholesterol and serum triglycerides in CCl4, Paracetamol and thioacetamide induced hepatotoxicity in rats to near normal levels. Histopathological observation exhibited the improved hepatic anatomy that is reversed the damage almost equal to standard drugs. The 70% methanolic extract of *Phyllanthus amarus* seeds in this study demonstrated antioxidant and protective activity.

Keywords: *Phyllanthus amarus* seeds, Hepatoprotective.

INTRODUCTION

Hepatotoxicity from drugs and chemicals is the commonest form of iatrogenic disease. Severity of hepatotoxicity is greatly increased if the drug is continued after symptoms develop. Among the various inorganic compounds producing hepatotoxicity are arsenic, phosphorus, copper and iron. The organic agents include certain naturally-occurring plant toxins such as pyrrolizidine alkaloids, mycotoxins and bacterial toxins. The synthetic groups of organic compounds are large number of medicinal agents. In addition, exposure to hepatotoxic compounds may be occupational, environmental or domestic that could be accidental, homicidal or suicidal ingestion. Acute toxicity of the *Phyllanthus amarus* seeds is evaluated as per Organization of Economic Cooperation and Development (OECD) guideline 423.[1, 2]

The specific objectives aimed in the present study are to prepare 70% methanolic extract of *Phyllanthus amarus* seeds using soxhlet extraction and cold maceration process and to assess organ protective role against experimentally induced Hepatotoxicity (CCl4, Paracetamol and Thioacetamide induced hepatotoxicity in Wister rats).[3, 4]

Phyllanthus amarus is a plant of the family Euphorbiaceae and has about approximately 800 species which are found in tropical and subtropical countries of the world. The name '*Phyllanthus*' means "leaf and flower" and named so because of its appearance where flower, fruit and leaf appears fused. *Phyllanthus amarus* is a branching annual glabrous herb which is 30-60 cm high and have slender, leaf-bearing branchlets, distichous leaves which are subsessile elliptic-oblong, obtuse, rounded base. Flowers are

yellowish, whitish or greenish, auxillary, males flowers in groups of 1-3 whereas females are solitary. Fruits are depressed-globose like smooth capsules present underneath the branches and seeds are trigonous, pale brown with longitudinal parallel ribs on the back. The plant has been found in Philippine, Cuba, Nigeria and among others. In India, *Phyllanthus amarus* is widely distributed as a weed in cultivated and waste lands.[5, 6]

MATERIALS AND METHODS

Plant material

The seeds of *Phyllanthus amarus* were collected from local gardens of Tirupati. The plant was identified and authenticated by Madhava Chetty, Department of Botany, Sri Venkateshwara University, Tirupati, Andhra Pradesh. A herbarium specimen is deposited in Nizam Institute of Pharmacy and Research Centre, Hyderabad, India.

Preparation of extracts

The crushed and dried seeds of *Phyllanthus amarus* were divided into two parts; one part was extracted successively with petroleum ether, benzene, chloroform and finally with methanol by soxhlet extraction and concentrated by rotary vacuum134. The other part is extracted by cold maceration process for aqueous extraction. The obtained extracts were dried by evaporation. The yield 18.05% w/w and 15.32% w/w were stored in refrigerator and weighed quantities were suspended in tween 80 and 2% tragacanth solution respectively, for the experiment. The extracts were used for In-vivo antioxidant studies. The samples were dried at room temperature, subsequently milled into powder and stored in air-tight stopped glassware before subjected to subject to preliminary qualitative phytochemical analysis.[7, 8]
The extracts were concentrated under reduced pressure and stored in a desicator until further use and the percentage yield of corresponding extracts were calculated.

Preliminary phytochemical screening

The preliminary phytochemical screening was carried out on the 70% methanolic extract of *Phyllanthus amarus* seeds for the presence of phytoconstituents. The tests for common phytochemicals were carried out by following standard methods.[9]

Experimental animals[10-14]

Healthy adult Wister rats, weighing 180-200g of either sex were used in this study. They were procured from Mahaveer agencies, Hyderabad. The animals were allowed to acclimatize for 7 days under laboratory conditions, before the experiment138. They were housed in polypropylene cages and maintained at 25˚C ± 2˚C under 12 hours dark / light cycle. The rats had free access to standard pellet chow (Gold Mohar Lipton India Ltd.) and water ad libitum throughout the experiment. They were housed in a cage of six animals per cage and were renewed thrice a week to ensure hygienity and maximum comfort for animals. The well maintained Nizam Institute of Pharmacy & Research Centre animal house held at Deshmukhi, Hyderabad was approved by CPCSEA bearing Reg. No. 1330/ac/10/CPCSEA. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee (IAEC) bearing Lr. No. IPRC/IAEC/PhD/2014/07. Prior to the beginning of the project work.

1. In vivo CCl4 induced hepatotoxicity

Lipid peroxidation, is accepted to be one of the principal cause of CCl4 induced liver injury, and is mediated by the production of free radical derivatives of CCl4.
Animals were randomly assigned into 5 groups of 6 individuals as pretreatment.
The study was carried out for 5 days.
Group-I Animals (-ve control) were administered 1ml tween 80 p.o.
Group-II Animals (+ve control) were administered CCl4 2 ml/kg i.p.
Group-III Animals (Standard) were administered silymarin 100 mg/kg p.o.
Group-IV Animals were administered methanolic extract 150 mg/kg p.o.
Group-V Animals were administered methanolic extract 250mg/kg p.o.
Group-1 received tween 80 (1 ml/kg) s.c., on 2nd and 3rd day. Group-II, III, IV and V received CCl4: tween 80 (1:1) at a dose of 2 ml/kg i.p., on 2nd and 3rd day, after 30 min of vehicle, 100 mg/kg silymarin, 150 mg/kg and 250 mg/kg methanolic extract of *Phyllanthus amarus* seeds administration. Animals were sacrificed on the 5th day under mild anesthesia. Hepatic tissues were collected and assessed.
The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation.
Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid.
Combine 1.0ml of biological sample (0.1 - 2 mg of membrane protein or 0.1-0.2 µmol) of lipid phosphate) with 2.0ml of TCA-TBA-HCl and mix thoroughly. The solution is heated for 15 min in boiling water both. After cooling, the

flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. Absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid. The malondialdehyde concentration of the sample can be calculated by using an extinction coefficient of 1.56X105M-1cm-1.

2. In vivo paracetamol induced hepatotoxicity

Lipid peroxidation, is accepted to be one of the principal cause of paracetamol induced liver injury, and is mediated by the production of free radical derivatives of paracetamol148.

Animals were randomly assigned into 5 groups of 6 individual's each. The study was done for 7 days.

Group-I Animals (-ve control) were administered 1 ml tween 80 p.o.

Group-II Animals (+ve control) were administered Paracetamol 2 g/kg p.o.

Group-III Animals (Standard) were administered silymarin 100 mg/kg p.o.

Group-IV Animals were administered methanolic extract 150 mg/kg p.o.

Group-V Animals were administered methanolic extract 250 mg/kg p.o.

On 5th day, after 30 min of normal saline, 100 mg/kg silymarin, 150 mg/kg and 250mg/kg methanolic extract of *Phyllanthus amarus* seeds administration to Group-II, III, IV and V respectively, received paracetamol 2 g/kg p.o., after 48 hours of paracetamol feeding, rats were sacrificed under mild ether anesthesia. Hepatic tissues were collected and assessed. The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation.

Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% w/v thiobarbituric acid; 0.25N hydrochloric acid. This solution may be mildly heated to assist in the dissolution of the thiobarbituric acid. Combine 1.0ml of biological sample (0.1-2.0 mg of membrane protein or 0.1-0.2 µmol) of lipid phosphate) with 2.0 ml of TCA-TBA-HCl and mix thoroughly. The solution is heated for 15 min in boiling water both. After cooling, the flocculent precipitate was removed by centrifugation at 1000 rpm for 10 min. the absorbance of the sample is determined at 535 nm against a blank that contains all the reagents minus the lipid.

The malondialdehyde concentration of the sample can be calculated by using an extinction coefficient of 1.56X105M-1cm-1.

3. In vivo thioacetamide induced hepatotoxicity

The animals were randomly assigned into 5 groups of 6 individuals each and the study was carried out for 9 days.

Group-I Animals (-ve control) were administered 1 ml tween 80 p.o.

Group-II Animals (+ve control) were administered thioacetamide100 mg/kg s.c.

Group-III Animals (Standard) were administered silymarin 100 mg/kg p.o.

Group-IV Animals were administered with methanolic extract 150 mg/kg p.o.

Group-V Animals were administered with methanolic extract 250 mg/kg p.o.

On 9th day, after 30 min of normal saline, 100 mg/kg silymarin, 150 mg/kg and 250 mg/kg methanolic extract of *Phyllanthus amarus* seeds administration to Group-II, III, IV and V respectively, received thioacetamide 100 mg/kg s.c., which was prepared in distilled water (2% solution). Food was withdrawn 12 hours before thioacetamide administration to enhance the acute liver damage in animals of group II, III, IV and V. The rats were sacrificed under mild ether anesthesia. Hepatic tissues were collected and assessed.

The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substance formation.

Histopathological Studies

Processing of isolated liver (Modified Luna's method 1960)

The animals were sacrificed and the liver was isolated and cut in to small pieces and preserved and fixed in 10% formalin for two days. Following this was the washing step where by the liver pieces were washed in running water for about 12 hrs. This was followed by dehydration with isopropyl alcohol of increasing strength (70%, 80% and 90%) for 12 hours each. Then the final dehydration is done using absolute alcohol with about three changes for 12 hours each. The cleaning was done by using chloroform with two changes for 15 to 20 minutes each. After clearing the liver pieces were subjected to paraffin infiltration in automatic tissue processing unit. The liver pieces were washed with running water to remove formalin completely. To remove the water, alcohol of increasing strengths was used since it is a dehydrating agent. Further alcohol was removed by using chloroform and chloroform removed by paraffin infiltration.

All the sections of the tissues were examined under microscope for analyzing the altered architecture of liver tissue due to CCl4, paracetamol and thioacetamide challenge and improved liver architecture due to pretreatment

with test extracts and standard (silymarin) drug.

Table 1: Effect of 70% methanolic extract of *Phyllanthus amarus* seeds on biochemical markers in CCl4 induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM						
	SGPT U/L	SGOT U/L	ALP U/L	Bilirubin mg/dl	Protein Mg/dl	Total Cholesterol Mg/dl	Triglycerides mg/dl
Negative control (1ml vehicle p.o)	55.55 ± 3.331	52.21± 5.617	122.29± 6.486	0.92± 0.029	8.88±0.34	110.88±10.771	171.22±7.198
Positive control CCl_4 + Tween 80 (1:1) 2 ml/kg, i.p	312.42± 14.275	318.41± 13.543	235.86± 8.207	4.89± 0.451	5.85±0.40	172.62±12.522	190.36±7.516
CCl_4 + Standard (Silymarin) 2 ml/kg, i.p + 100 mg/kg, p.o	65.39±8.700***	71.21± 6.843**	95.68± 9.485***	1.54± 0.301**	8.46± 0.24***	120.25±8.980*	145.48±8.253*
CCl_4 + 70% methanolic extract 2 ml/kg, i.p + 150 mg/kg, p.o	124.77 ± 5.319**	108.96± 2.597**	131.62± 10.462**	2.47± 0.188**	6.74± 0.56*	137.11±9.494	177.99±7.207
CCl_4 + 70% methanolic extract 2 ml/kg, i.p + 250 mg/kg, p.o	77.84±3.083***	72.69± 5.352**	97.44± 7.425***	1.20± 0.190**	7.92± 0.33**	118.53±7.185*	175.79±6.406*
CCl_4 + aqueous extract 2 ml/kg, i.p + 300 mg/kg, p.o	130.76 ±5.319**	100.54± 2.597**	131.62± 10.462**	1.48± 0.188**	6.25±0.51*	150.26±9.494	170.57±7.207

Values are the mean ± SEM of six rats / treatment
Significance **P<0.01 and ***P<0.001 compared to CCl_4 treatment

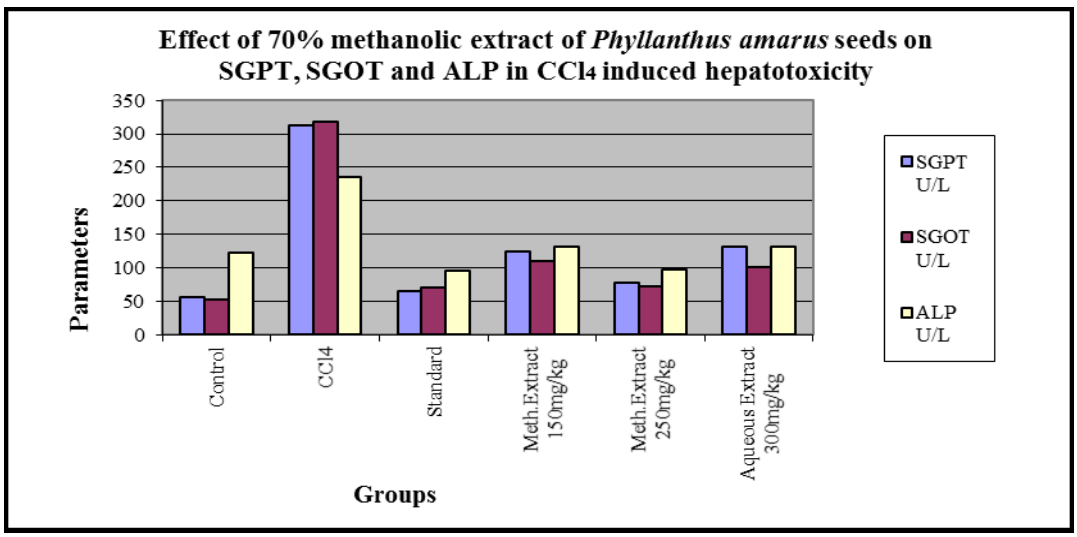
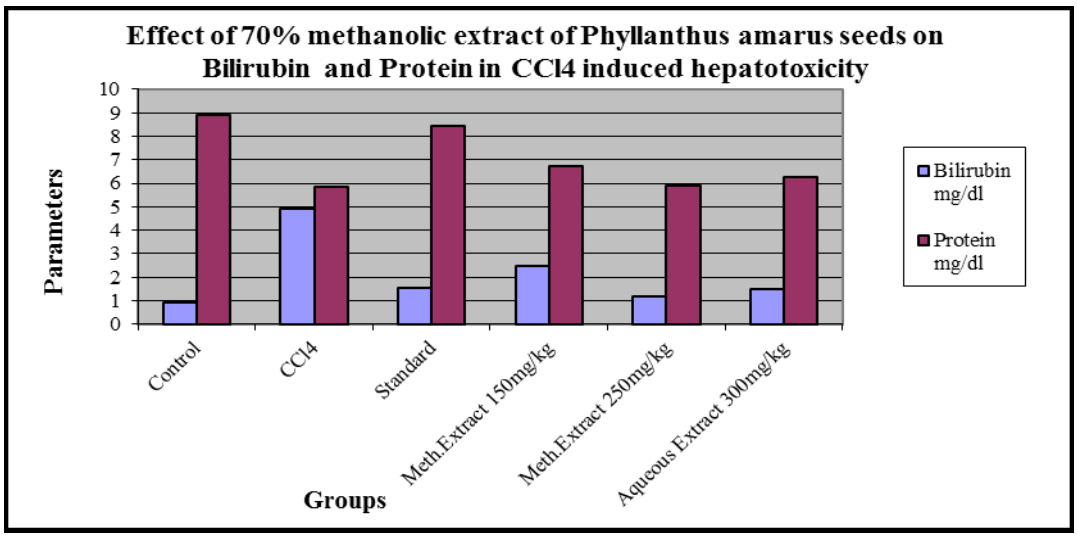


Fig. 1:



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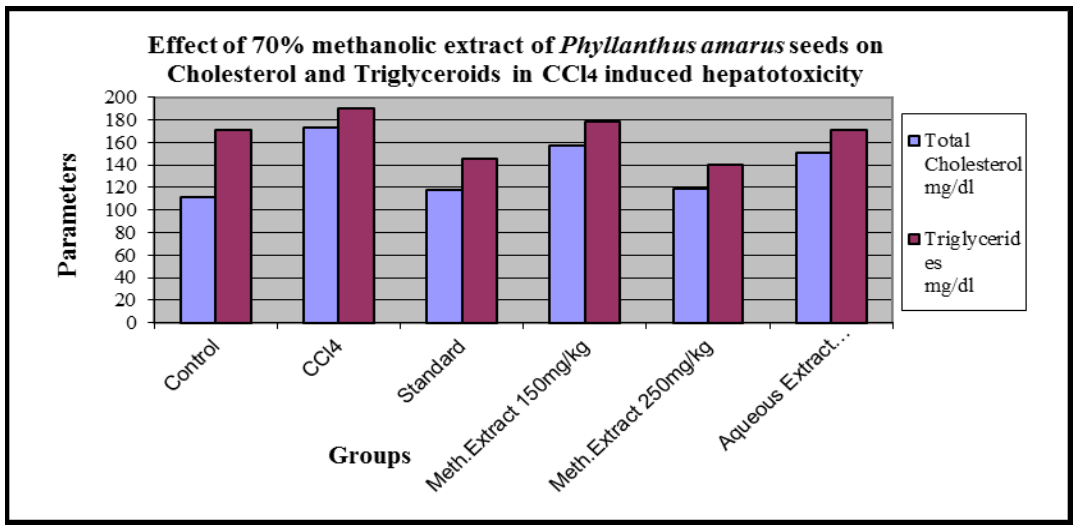


Fig. 3:

Effect of 70% methanolic extract of *Phyllanthus amarus* seeds on biochemical markers in paracetamol induced hepatotoxicity:

RESULTS

There was increased level of SGPT, SGOT and ALP observed in paracetamol treated groups (263.17 U/L, 323.51 U/L and 425.36 IU/L respectively). The extract showed a dose dependent effect. SGPT levels was restored to 80.36 U/L, SGOT levels was restored to 98.59 U/L and ALP levels was restored to 172.86 U/L by 250 mg/kg 70% methanolic extract of the seeds which was near to the effect of 100mg/kg silymarin i.e. 78.56 U/L, 93.47 U/L and 169.93 U/L respectively.

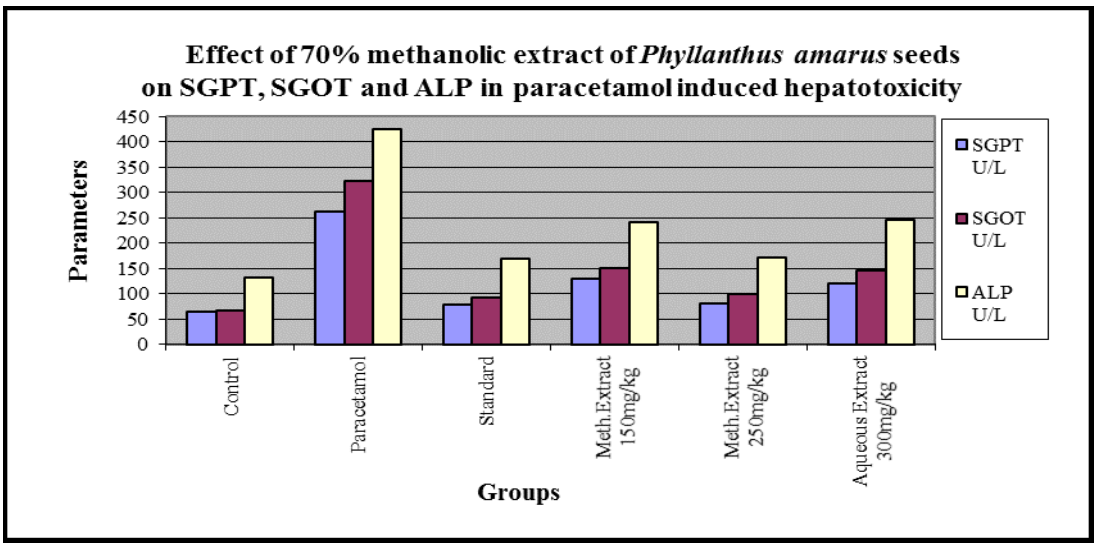
In case of the total bilirubin and protein, a dose dependent effect of the extract was observed. 250 mg/kg 70% methanolic extract reduced the elevated levels of total bilirubin i.e. from 4.21 mg/dl to 1.08 mg/dl with compared to standard 1.09 mg/dl. The protein levels were restored back from 5.85 mg/dl to 7.92 mg/dl which is almost equal to 100 mg/kg standard silymarin 8.46 mg/dl.

There was no significant rise in total cholesterol and triglyceride levels in paracetamol treated group. Dose dependent effect was observed with the 70% methanolic extract and of 250 mg/kg 70% methanolic extract was comparable with 100 mg/kg silymarin.

Table 2: Effect of 70% methanolic extract of *Phyllanthus amarus* seeds on biochemical markers in paracetamol induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM						
	SGPT U/L	SGOT U/L	ALP U/L	Bilirubin mg/dl	Protein Mg/dl	Total Cholesterol Mg/dl	Triglycerides mg/dl
Negative control (1ml vehicle p.o)	64.69± 5.088	67.16± 3.753	131.68± 3.143	0.89±0.077	8.88±0.34	115.63±5.406	175.7±5.670
Positive control (Paracetamol) + Tween 80 (1:1) 2 g/kg, p.o	263.17 ± 6.908	323.51± 8.251	425.36± 8.282	4.21±0.058	6.88±0.40	185.36±4.106	210.56±7.688
Paracetamol + Silymarin 2 g/kg, p.o + 100 mg/kg, p.o	78.56± 6.230***	93.47± 4.525***	169.93± 4.433***	1.09± 0.064***	8.96± 0.24**	118.67± 6.235***	179.17± 8.888**
Paracetamol + 70% meth. extract 2 g/kg, p.o + 150 mg/kg, p.o	129.79± 1.687***	151.36± 0.756**	241.49± 1.006***	1.38±0.126***	6.74±0.56*	146.49± 0.757*	192.77±1.474*
Paracetamol + 70% meth. extract 2 g/kg, p.o + 250 mg/kg, p.o	80.36± 1.022***	98.59± 0.928**	172.86± 0.969***	1.08± 0.430***	7.90± 0.33**	121.27± 1.332**	181.09±0.913
Paracetamol + aqueous extract 2 g/kg, p.o + 300 mg/kg, p.o	121.61± 1.679***	145.96± 0.725**	245.49± 1.065***	1.27± 0.116***	6.25±0.51*	139.42± 0.702*	185.85±1.972*

Values are the mean ± SEM of six rats / treatment
Significance *P<0.05, **P<0.01 and ***P<0.001 compared to paracetamol treatment



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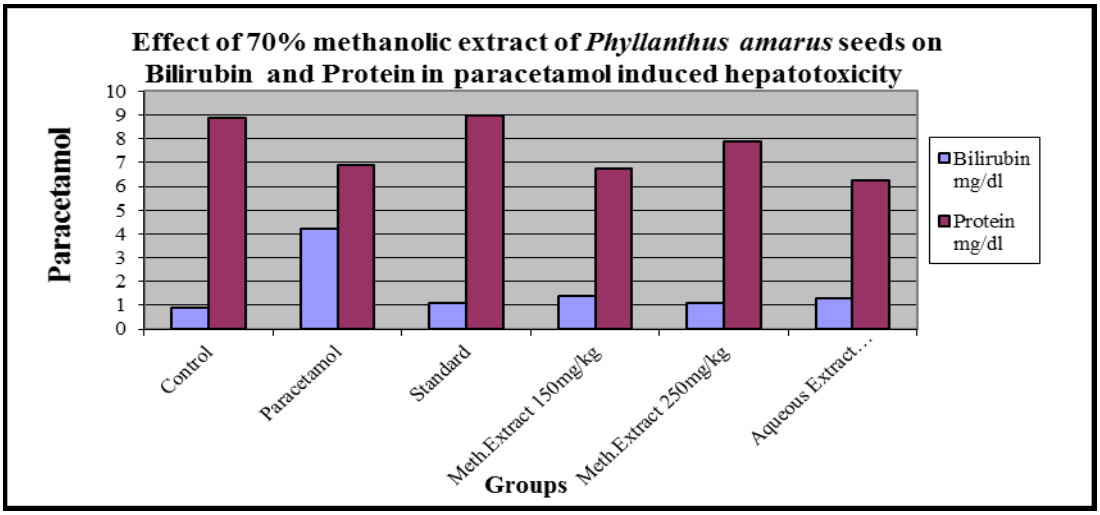

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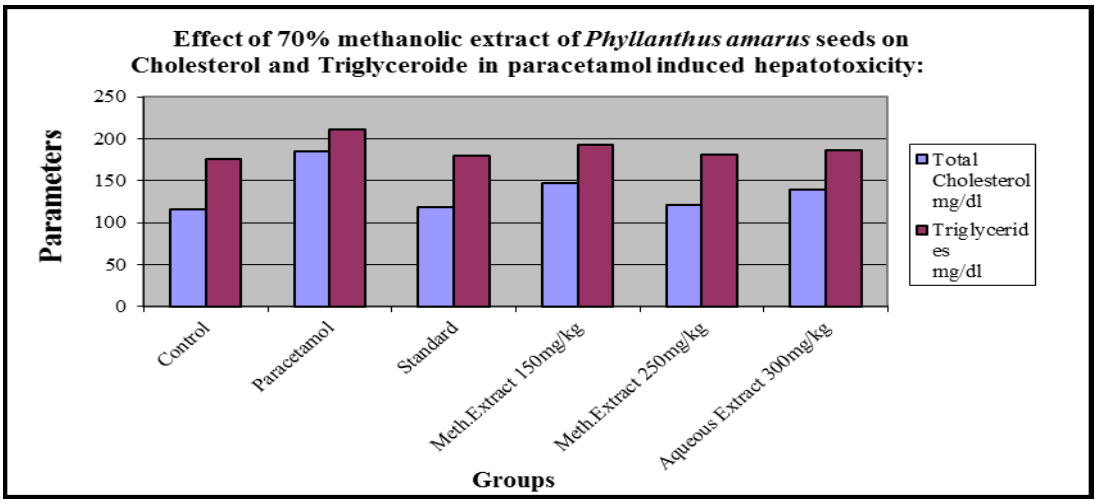


Fig. 6:

Effect of 70% methanolic extract of *Phyllanthus amarus* seeds on biochemical markers in Thioacetamide induced hepatotoxicity:

RESULTS

There was increased level of SGPT, SGOT and ALP observed in thioacetamide treated groups (299.58 U/L, 403.35 U/L and 469.35 IU/L respectively). The extract showed a dose dependent effect. SGPT levels was restored to 101.53 U/L, SGOT levels was restored to 108.70 U/L and ALP levels was restored to 149.70 U/L by 250 mg/kg 70% methanolic extract of the seeds which was near to the effect of 100 mg/kg silymarin i.e. 98.33 U/L, 106.13 U/L and 146.62 U/L respectively.

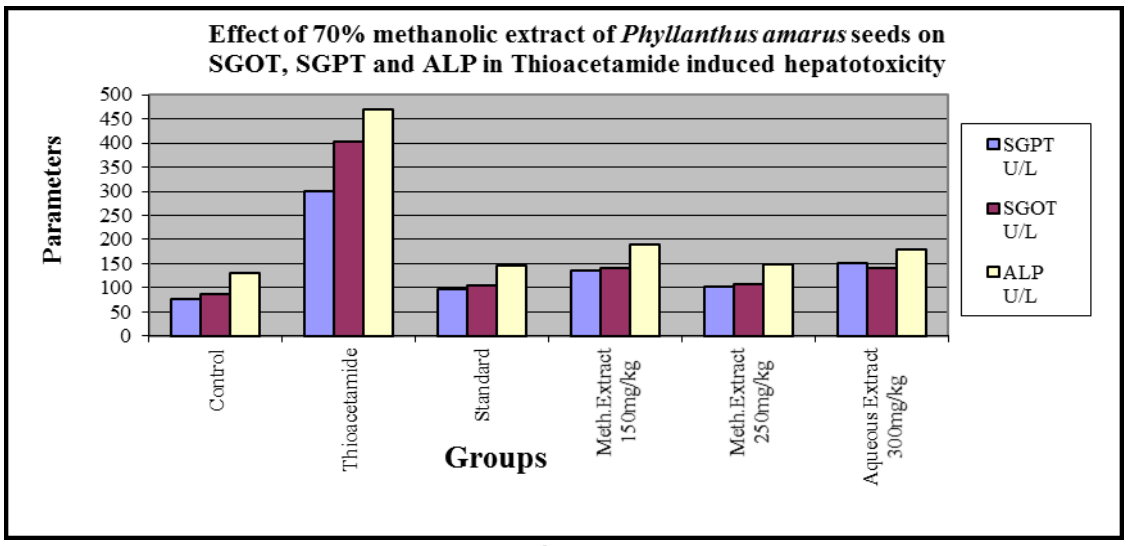
In case of the total bilirubin and protein, a dose dependent effect of the extract was observed. 250mg/kg 70% methanolic extract reduced the elevated levels of total bilirubin i.e. from 2.38 mg/dl to 1.03 mg/dl with compared to standard 1.05 mg/dl. The protein levels were almost reduced from 5.41 mg/dl to 5.52 mg/dl with that of 100 mg/kg standard silymarin 8.5 mg/dl.

There was no significant rise in total cholesterol and triglyceride levels in thioacetamide treated group. Dose dependent effect was observed with the 70% methanolic extract and of 250 mg/kg 70% methanolic extract was comparable with 100 mg/kg silymarin.

Table 3: Effect of 70% methanolic extract of *Phyllanthus amarus* seeds on biochemical markers in Thioacetamide induced hepatotoxicity

Treatment	Biochemical parameters Mean ± SEM						
	SGPT U/L	SGOT U/L	ALP U/L	Bilirubin mg/dl	Protein Mg/dl	Total Cholesterol Mg/dl	Triglycerides mg/dl
Negative control (1ml vehicle)	75.78± 2.818	87.38± 6.121	129.68± 5.013	0.93±0.047	8.88±0.34	112.74± 3.585	176.94±3.667
Positive control (Thioacetamide) + Tween 80 (1:1) 100mg/kg, s.c	299.58± 10.722	403.35± 8.667	469.35± 7.218	2.38±0.116	5.41±0.40	190.54± 11.128	215.87±10.264
Thioacetamide + Standard (Silymarin) 2 ml/kg, s.c + 100 mg/kg, p.o	98.33± 4.043***	106.13± 6.927***	146.62± 7.408*	1.05± 0.061***	8.35±0.24**	125.28± 5.656***	174.37± 9.903**
Thioacetamide + 70% meth. extract 2 ml/kg, s.c + 150 mg/kg, p.o	134.67± 1.069***	141.49± 1.198**	190.77± 1.108**	1.25± 0.052***	6.73±0.56	156.08± 0.954**	191.63±0.595
Thioacetamide + 70% meth. extract 2 ml/kg, s.c + 250 mg/kg, p.o	101.53± 0.667***	108.56± 1.134**	149.70± 0.905**	1.03± 0.025***	5.52±0.33**	129.31± 0.981**	177.49±0.815*
Thioacetamide + aqueous extract 2 ml/kg, s.c + 300 mg/kg, p.o	150.67± 1.168***	140.09± 1.108**	180.68± 1.108**	1.19± 0.060***	6.25±0.52*	166.23± 0.950**	187.63±0.655

Values are the mean ± SEM of six rats / treatment
Significance *P<0.05, **P<0.01 and ***P<0.001 compared to thioacetamide treatment



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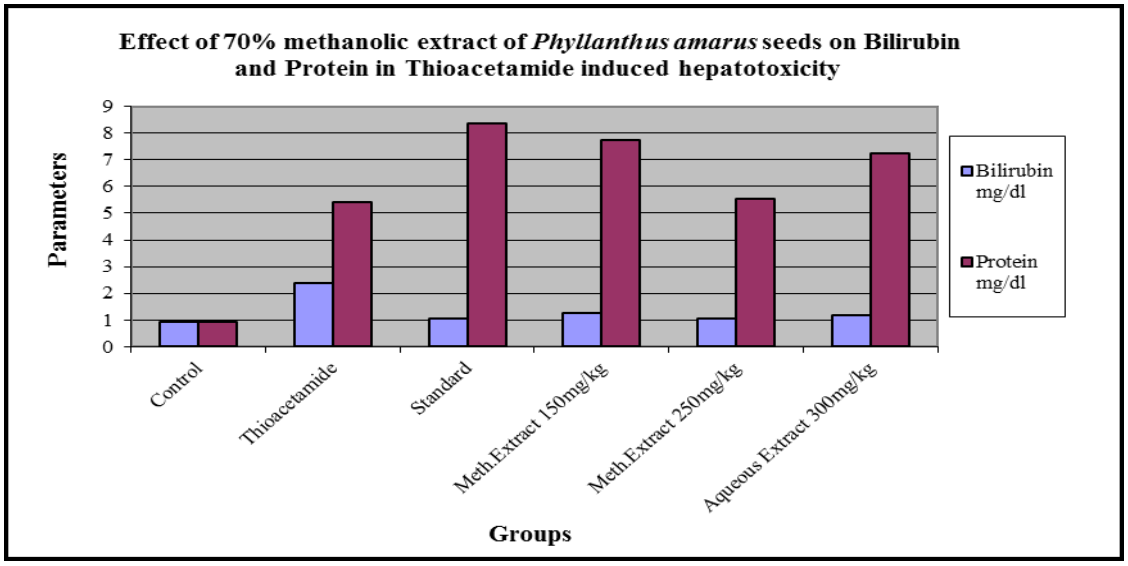

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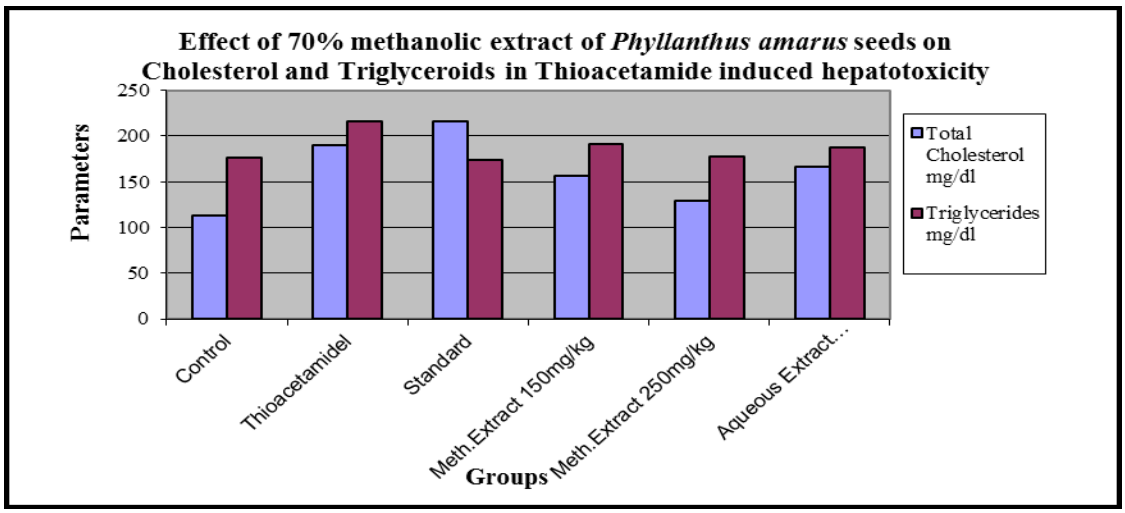

Fig. 9:

Histopathological studies in CCl4 induced hepatotoxicity

RESULT

Group A: (Normal liver). In the case of normal control, hepatic globular structure central vein, portal traid and kupffer cells look normal.

Group B: (Necrotic liver). In the case of CCl4 treated group, hepatic cells have shown moderate degree of fatty degeneration and ballooning of hepatocytes, fatty cyst and infiltration of lymphocytes and proliferation of kupffer cells. Liver sinusoids were congested. Centri-lobular necrosis was observed.

Group C: (Regenerative changes in liver). In the case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. There were occasional fatty cells and few cells have shown hyaline globule in the cytoplasm. There were occasional areas of lymphocytic infiltration and kupffer cell proliferation.

Group D: (Light regeneration of hepatocytes). In the case of 150 mg/kg methanolic extract group the hepatic globular architecture was normal. A few areas show lymphatic infiltration. Majority of hepatocytes were normal.

Group E: (Regeneration of hepatocytes). In the case of 250 mg/kg methanolic extract group the hepatic architecture was maintained. Areas of kupffer cells proliferation and sinusoids appeared to be normal.

Group F: (fatty liver).In the case of 300 mg/kg aqueous extract group, the hepatic globular architecture was normal. Hepatic cells have shown moderate degree of fatty degeneration of hepatocytes and centri lobular inflammatory cells.

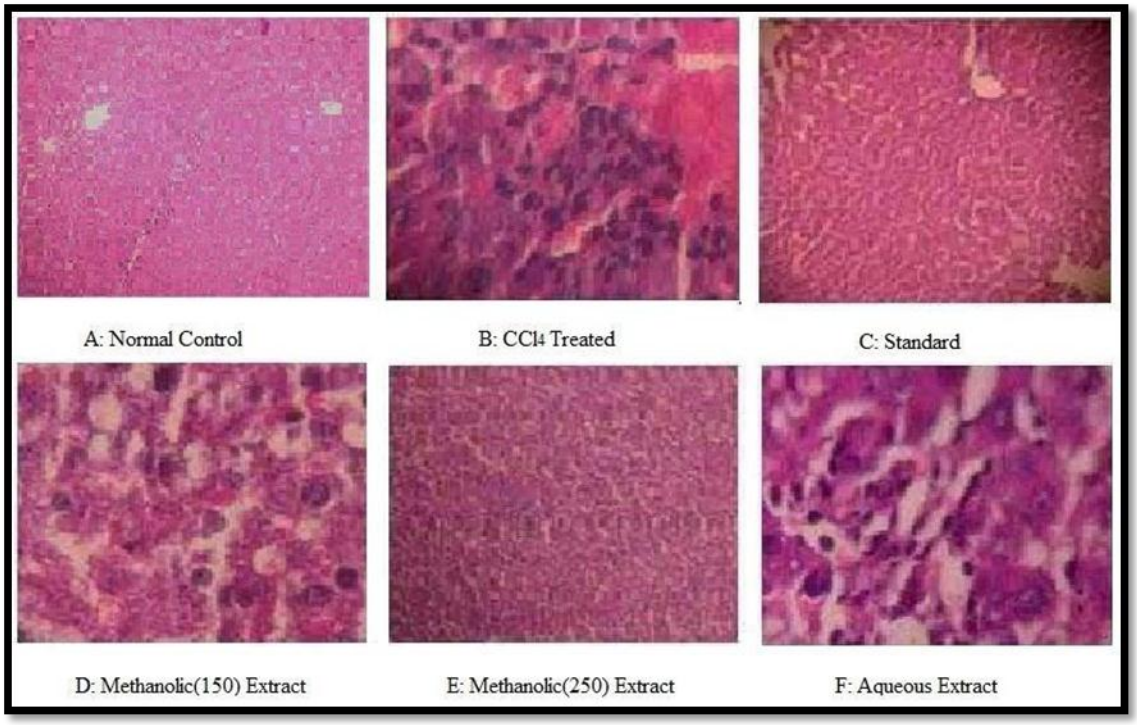

Fig. 4: Histopathological studies in CCl4 induced hepatotoxicity

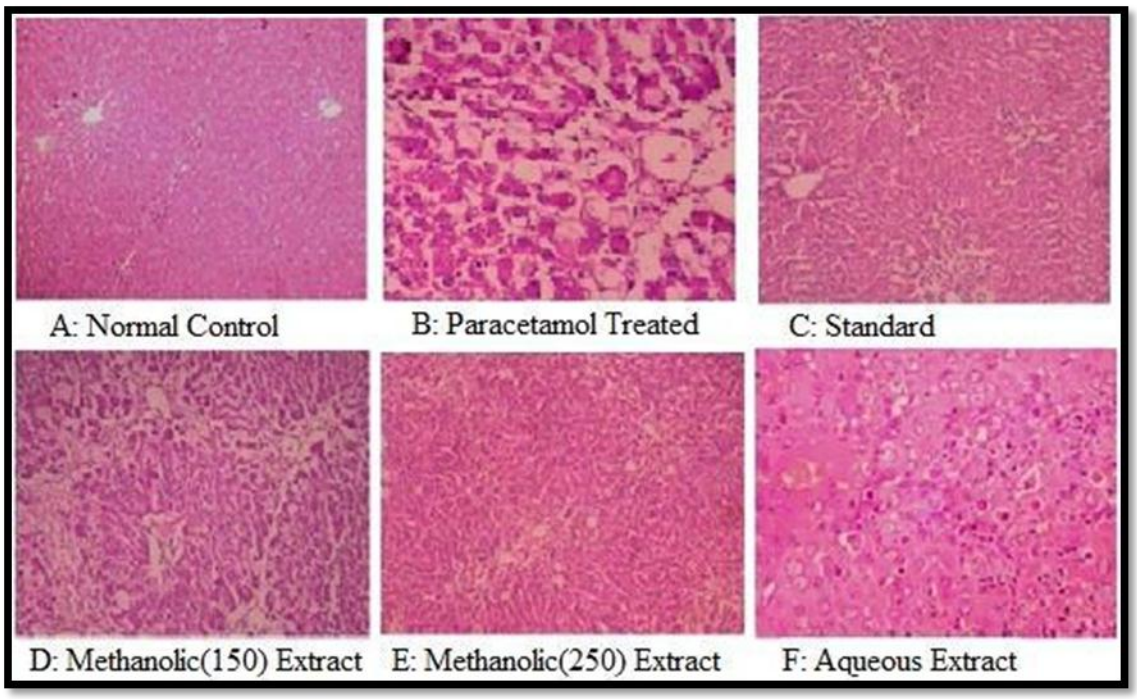

Fig. 5: Histopathological studies in Paracetamol induced hepatotoxicity

Histopathological studies in Paracetamol induced hepatotoxicity Result

Group A: (Normal liver). In the case of normal control, hepatic globular structure central vein, portal traid and kupffer cells look normal.

Group B: (Necrotic liver). In the case of CCl4 treated group, hepatic cells have shown necrotic cells around the central vein, fatty changes and inflammatory cells.

Group C: (Regenerative changes in liver). In the case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. There were macrophage infilteration and improvement of histological appearance with less evidence of necrosis.

Group D: (Light regeneration of hepatocytes). In the case of 150 mg/kg methanolic extract group the hepatic globular architecture was

normal. There were occasional areas of lymphocytic infiltration and kupffer cell proliferation.
Group E: (Regeneration of hepatocytes). In the case of 250 mg/kg methanolic extract group the hepatic architecture was maintained. Regenerated tubular epithelium and healing of necrotic changes.
Group F: (fatty liver).In the case of 300 mg/kg aqueous extract group, the hepatic globular architecture was normal. Hepatic cells have shown moderate degree of fatty degeneration of hepatocytes.

Histopathological studies in thioacetamide induced hepatotoxicity
Result
Group A: (Normal liver). In the case of normal control, hepatic globular structure central vein, portal traid and kupffer cells look normal.
Group B: (Necrotic liver). In the case of CCl4 treated group demonstrating the destruction of architectural pattern, nodule formation in the lobular zone, inflamed periportal zone, moderate inflammation of portal area.
Group C: (Regenerative changes in liver). In the case of 100 mg/kg silymarin treated group the hepatic globular architecture was normal. Regenerative changes in normal hepatocytes.
Group D: (Light regeneration of hepatocytes). In the case of 150 mg/kg methanolic extract group the hepatic globular architecture was normal. A few areas show lymphatic infiltration. Majority of hepatocytes were normal.
Group E: (Regeneration of hepatocytes). In the case of 250 mg/kg methanolic extract group the hepatic architecture was maintained with regeneration of hepatocytes.
Group F: (fatty liver).In the case of 300 mg/kg aqueous extract group, the hepatic globular architecture was normal. Hepatic cells have shown normal lobular architecture no necrosis or fatty changes or any inflammatory reaction.

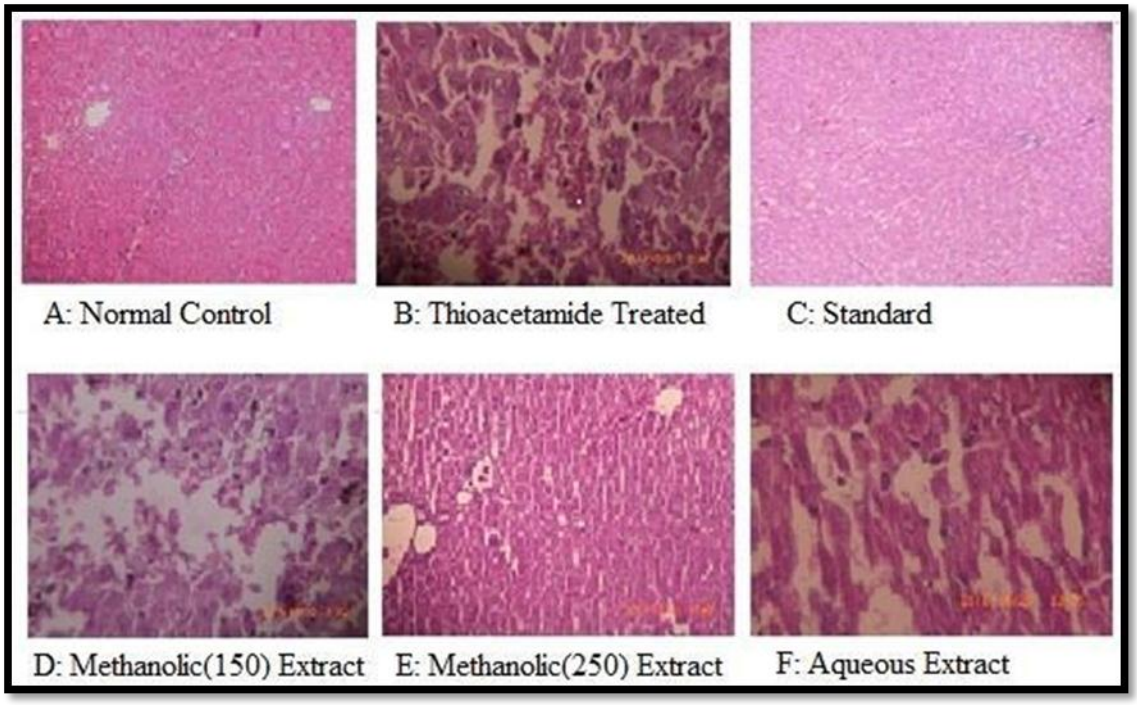

Fig. 6: Histopathological studies in thioacetamide induced hepatotoxicity

DISCUSSION

The herbs containing active principles are reported to be highly effective in preventing or curing the liver and kidney toxicities due to the above mentioned challenges. In concurrent with this, in the present study the herbs by name *Phyllanthus amarus* containing polyphenolic compounds was selected to asses antioxidants and organ protective potential.

In this study the variation of phyllanthin in Hexane: Ethyl acetate (2:1) gave good resolution of phyllanthin from other closely related lignans. The HPTLC method for the quantitative estimation of Phyllanthin was validated with regard to their specificity, precision, accuracy and linearity. Quantification of the marker may also reflect the quality of raw material in general and quantitative composition of other phytoconstituents in particular.

The 70% methanolic extract of seeds of *Phyllanthus amarus* has shown significant and dose dependant superoxide anion scavenging, hydroxyl radical scavenging, nitric oxide anion scavenging and reducing power. In addition, treatment with 70% methanolic extract 250 mg/kg seeds significantly restored the tissue GSH and reduced the lipid peroxidation. These results are indicating that the seeds also possess antioxidant property. Therefore the 70% methanolic extract 250 mg/kg seeds can be used as an organ protective potential against xenobiotics induced hepatotoxicity and nephrotoxicity in rats.

Three experimental models of hepatotoxicity (CCl4, paracetamol, thioacetamide induced in hepatotoxic model in rats) and three models of nephrotoxicity (cisplatin, gentamycin, paracetamol induced in rats) was used to assess the organ protective potential of 70% methanolic extracts and aqueous extract of the seeds.

Since our extract has demonstrated significant free radical scavenging activity, prevented the depletion of GSH to significant extent and significantly reduced the lipid peroxidation. Due to its antioxidant potential, our study does not rule out the possibility of inhibition of CYP4502E1 even resulting in the prevention of generation of trichloromethyl radical.

Paracetamol 2 mg/kg dose has induced hepatotoxicity as indicated by the elevation in the above mentioned biochemical markers. Further this is confirmed by histopathological observation. Histopathological observation revealed that there are sites of lymphocytic infiltration, ballooning of hepatocytes, congestion of sinusoids and histopathological parameters of hapatotoxicity in paracetamol treated group indicated that hepatotoxicity is induced by paracetamol.

Treatment with 250 mg/kg 70% methanolic extract of seeds reduced the elevated biochemical markers of hepatotoxicity. Even histopathologically it was found that the areas of kupffer cell proliferation, site of lymphocytic infiltration, sinusoids centrilobular necrosis, were reduced. Sign of hepatic regeneration observed. These observations are indicating that 250 mg/kg 70% methanolic extract of seeds possess hepatoprotective properly is these model.

In the present study 250 mg/kg 70% methanolic extract of seeds has significantly prevented the depletion of GSH, reduced the lipid peroxidation and has shown significant and dose dependent antioxidant property. The heptoprotective activity of the 250 mg/kg 70% methanolic extract of seeds assigned to antioxidant property.

Thioacetamide 100 mg/kg elevated the biochemical markers of hepatotoxicity. Lymphocytic infiltration, ballooning of hepatocyte, congestion of sinusoids and arteriole, kupffer cell proliferation, centrilobular necrosis was observed histopathologically. Both biochemical and histopathological observation are indicating that thioacetamide induces hepatotoxicity in rats. These findings are inconformity with earlier report.

Treatment with to 250 mg/kg 70% methanolic extract of seeds reduced the elevation biochemical markers of hepatotoxicity. Even reversed the histopathological observation followed. There are reports that thioacetamide is a potent hepatotoxic. The hepatotoxicity induced by the liver is due to the production of its metabolite thioacetamide-5-oxide, which is directly acts on various cellular components and destroys it.

In the present study 70% methanolic extract of the seeds has prevented the depletion of tissue GSH and decreased the lipid peroxidation and has demonstrated dose dependent invitro antioxidant property. Therefore, even in this model also hepatoprotective property may be due to the antioxidant principles of the test extract.

In the paracetamol and thioacetamide model the elevation of level of SGOT was more pronounced when compared to SGPT. This may be due to the fact that SGOT also present in nephrons and there are reports that higher doses of paracetamol also cause nephrotoxicity, which may results in the leakage of SGOT in the serum. This leakage of SGOT from nephron into serum may be the cause for the more pronounced level of SGOT, however further studies are needed to confirm.

CONCLUSION

The 70% methanolic extract demonstrated the dose dependent antioxidant activity. Treatment with 70% ethanolic extract has protected the liver from CCl4, paracetamol and thioacetamide induced hepatotoxicity. This was marked by reducing the elevated levels of biochemical markers like SGPT, SGOT, ALP, bilirubin, protein, cholesterol and triglycerides. In addition, histopathological observation has shown that there is an improvement in the architecture of liver.

Treatment with 70% methanolic extract has brought back the elevated levels of SGPT, SGOT, ALP, bilirubin, protein, total cholesterol and triglycerides in CCL4, paracetamol, thioacetamide induced hepatotoxicity in rat to near normal levels.

Histopathological studies supplemented the findings by showing mild hepatic degeneration with absence of necrosis in comparison with the standard and control. Thus indicating the prominent significance of 70% methanolic extract 250 mg/kg of *Phyllanthus amarus* seeds in hepatoprotection against CCL4, paracetamol and thioacetamide induced hepatotoxicity.

This study only revealed the usefulness of *Phyllanthus amarus* seeds in case of hepatic and renal disease. As the plants phytochemical and pharmacological data is covered, so there is lot more scope for further molecular study to know the exact mechanism of each active principal responsible for hepatoprotective activity of plant.

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