

Hepatoprotective and nephroprotective activity of *Phyllanthus amarus* Schum & Thonn. seed extract

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Received for publication September 15, 2012; accepted November 10, 2012

Abstract

Conventional drugs used in the treatment of liver and kidney diseases are sometimes inadequate and can lead to serious side effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver and kidney diseases in order to replace currently used drugs of doubtful efficacy and safety. Leaves of *Phyllanthus amarus* Schum & Thonn (Family: Euphorbiaceae) is traditionally known for its liver and kidney protection. We have made an attempt to find out the hepatoprotective and nephroprotective activity on *Phyllanthus amarus* seeds.

Hepato and nephrotoxicity was induced by thioacetamide (100 mg/kg s.c) and gentamycin (80 mg/kg i.p), respectively. *Phyllanthus amarus* seed extracts (70% methanolic 250 mg/kg and aqueous 300 mg/kg) were administered orally to Wistar rats. Hepatoprotection was assessed by measuring the biochemical parameters like serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total bilirubin, direct bilirubin, triglycerides, cholesterol and histopathological changes in liver. Whereas nephroprotection was assessed by measuring serum creatinine, blood urea nitrogen (BUN) and kidney weight.

Histopathological analysis of liver samples also confirmed the hepatoprotective activity of methanolic extract of the seeds, which was comparable to the standard silymarin. The results obtained in this study indicate that the methanolic extract has significant effect than aqueous extract when compared to silymarin and cystone, respectively. The mechanism involved in the protection could be associated with its strong capability to reduce the enhanced levels of above parameters. Hence, the *Phyllanthus amarus* seed possesses a potent protective effect against thioacetamide-induced hepatic damage, and gentamycin-induced renal damage.

Key words: *Phyllanthus amarus*, Thioacetamide, Gentamycin, Hepatoprotective, Nephroprotective

Introduction

The use of natural remedies for the treatment of liver diseases, has a long history, starting with the Ayurvedic treatment, and extending to the Chinese, European and other systems of

traditional medicines. The 21st century has seen a paradigm shift towards therapeutic evaluation of herbal products in liver and kidney diseases by carefully synergizing the strengths of the traditional systems of medicine with that of the modern concept of evidence-based medicinal evaluation, standardization of herbal products and randomized placebo controlled clinical trials to support clinical efficacy (Thyagarajan *et al.*, 2002).

Liver diseases and kidney failure have become one of the major causes of morbidity and mortality in man and animals all

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over the globe. Hepatotoxicity and nephrotoxicity due to drugs appear to be the most common contributing factors. Many investigators have turned to simpler experimental models for studying drug metabolism and response of the organ or liver cells to potentially toxic agents (Pearson and Wienkers, 2009). Conventional drugs used in the treatment of liver diseases are sometimes inadequate and can have serious side effects. Therefore, it is necessary to search for alternative drugs for the treatment of liver disease in order to replace currently used drugs of doubtful efficacy and safety (Ali *et al.*, 2009). Steroids vaccines and antiviral drugs that are employed as therapy for liver diseases have potential adverse effects, especially when administered for long periods. There is worldwide trend for use of traditional herbal drugs for the treatment of liver diseases. Several plant sources have been found as potential hepato and nephroprotective agents with diverse chemical structures.

In the absence of a reliable liver protective drug in modern medicine, there are a number of medicinal preparations in Ayurveda, recommended for the treatment of liver disorders. Due to severe undesirable side effects of synthetic agents, there is growing focus to follow systematic research methodology and to evaluate scientific basis for the traditional herbal medicines that are claimed to possess hepatoprotective activity. A single drug cannot be effective for all types of severe liver diseases (Shahani, 1999). Drugs, diagnostic agents and chemicals are well known to be nephrotoxic. Nephrotoxic agents can produce damage either by directly reacting with cellular macromolecules and membrane components or from metabolism within the tubular cells to toxic products. These toxic metabolites mainly include free radicals (Best and Taylors, 1984). Therefore, an effective formulation has to be developed, using indigenous medicinal plants, with proper pharmacological experiments and clinical trials.

The organ protective property was screened against thioacetamide induced hepatotoxicity and gentamycin induced nephrotoxicity model in rats, were used to assess the organ toxicity potential. The seeds of the plant were reported to possess lignans and related flavonoids which are known antioxidants. Therefore, the *Phyllanthus amarus* Schum & Thonn. (Family: Euphorbiaceae) seeds were selected for assessing antioxidant and organ protective potential. However, till date no scientific evaluations are conducted on seeds for confirming its role as hepatoprotective and nephroprotective agent. Thus, the present study is designed to investigate the activities of *Phyllanthus amarus* seed extracts on hepatotoxicity and nephrotoxicity in rat models.

The extract of *Phyllanthus amarus* seeds was subjected for the following studies:

- (i) Acute toxicity studies
- (ii) Evaluation of hepatoprotective activity (Standard silymarin)

- Thioacetamide-induced hepatotoxicity
- Liver biochemical markers: SGOT, SGPT, ALP, total and direct bilirubin, cholesterol, triglyceroides

(iii) Evaluation of nephroprotective activity

- Gentamycin-induced nephrotoxicity (Standard cystone)
- body weight
- Kidney biochemical markers
- Serum creatinine, blood urea

Materials and Methods

Plant material

The seeds of *Phyllanthus amarus* Schum & Thonn. were collected from local gardens of Tirupati. The plant was identified and authenticated by Dr. 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 vacuum (Diener *et al.*, 1995). The other part is extracted by cold maceration process for aqueous extraction (William *et al.*, 1971). The obtained extracts were dried by evaporation. The yield 30% w/w and 15.6% 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 its hepatoprotective and nephroprotective activity.

Experimental animals

The 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 experiment (Obianime, and Oche, 2008). 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 hygiene and maximum comfort for animals. Ethical clearance for handling the animals was obtained from the Institutional Animals Ethical Committee (IAEC Registration No. 1330/ac/10/CPCSEA), prior to the beginning of the work.

Acute toxicity studies

Healthy adult Wister rats were subjected for oral toxicity study. The animals were overnight fasted and divided into two groups, each contains three animals. They were fed with 70%

methanolic and aqueous extracts (Ghosh, 1985). The animals were observed continuously for 2 hours for behavioral, neurological and autonomic profiles and after 24 and 72 hours for any lethality (Turner, 1965).

Experimental procedures

Table 1: Evaluation of hepatoprotective and nephroprotective activity

Groups	Administration	Hepatoprotective activity	Nephroprotective activity
Group I	Negative control	Tween 80 (1 ml/kg p.o.)	Tween 80 (1 ml/kg p.o.)
Group II	Positive control	Thioacetamide 100 mg/kg s.c.	Gentamycin 80 mg/kg i.p.
Group III	Standard	Silymarin 100 mg/kg p.o.	Cystone 5 ml/kg p.o.
Group IV	Methanolic extract	250 mg/kg p.o.	250 mg/kg p.o.
Group V	Aqueous extract	300 mg/kg p.o.	300 mg/kg p.o.

Hepatoprotective activity

Wister rats were randomly assigned into 5 groups of 6 individuals each, the study has carried out for 9 days (Chattopadhyay, 2003).

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

Group-II: Animals (+ve control) were administered thioacetamide 100 mg/kg s.c.,

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

Group-IV: Animals were administered with methanolic extract 250 mg/kg p.o.,

Group-V: Animals were administered with aqueous extract 300 mg/kg p.o.,

On 9th day after 30 min of vehicle, 100 mg/kg silymarin, 250 mg/kg 70% methanolic extract and 300 mg/kg aqueous 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 tween 80 (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.

Blood samples were collected for evaluating the serum biochemical parameters and liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of hepatic architecture in each group microscopically. The blood so collected was centrifuged immediately to get clear serum and was subjected to various biochemical studies.

Estimation of serum SGPT and SGOT

Method: 2, 4-DNPH (Reitman and Frankel, 1957)

Working reagent preparation

Reagent 1, 2 and 4 are ready to use.

Solution 1: Dilute 1ml of reagent 3 to 10ml with purified water

Procedure

Pipette into tube marked	Blank	Standard	Test	Control
Volume in ml.				
Reagent 1	0.25	0.25	0.25	0.25
Serum	-	-	0.05	-
Standard	-	0.05	-	-
Mix well and incubate at 37°C for 30 minutes				
Reagent 2	0.25	0.25	0.25	0.25
Deionised water	0.05	-	-	-
Serum	-	-	-	0.05
Mix well and allow to stand at incubate at 37°C for 30 minutes				
Solution 1	2.5	2.5	2.5	2.5

Mix well and read the OD against purified water in a photometer at 505 nm, within 15 minutes.

$$\text{SGPT activity (IU/L)} = \frac{\text{Abs. test} - \text{Abs. control} \times \text{Cone. of std.}}{\text{Abs. std} - \text{Abs. blank}}$$

Estimation of alkaline phosphate

Method: (Kind and King's, 1959)

Preparation of working solution

	Blank (B)	Standard (S)	Test (T)	Control (C)
Working buffered substrate	0.5 ml	0.5 ml	0.5 ml	0.5 ml
Purified water	1.5 ml	1.5 ml	1.5 ml	1.5 ml
Mix well and incubate at 37°C for 3 minutes				
Serum	-	-	-	0.05 ml
Reagent 3	-	0.05 ml	-	-
Mix well and incubate at 37°C for 3 minutes				
Reagent 2	1.0 ml	1.0 ml	1.0 ml	1.0 ml
Serum	-	-	0.05 ml	-

All the volumes mentioned were adjusted proportionately, depending on cuvette capacity. O.D. at 510 nm is measured.

$$\text{Serum ALP activity in KA units} = \frac{\text{O.D test} - \text{O.D control}}{\text{O.D std} - \text{O.D blank}} \times 10$$

Nephroprotective activity

Five groups of six rats in each were used in this model. They were weighed individually on first and eighth day of treatment (Vijay Kumar *et al.*, 2000).

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

Group-II : Animals (+ve control) were administered with gentamycin 80 mg/kg i.p.

Group-III: Animals (Standard) were administered with cystone 5 ml/kg p.o.

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

Group-V : Animals were administered with aqueous extract 300 mg/kg p.o.

Group I received only normal saline throughout the course of the experiment, was used as control. Group II animals received daily i.p. injection of gentamycin (80 mg/kg b.w.) for eight days. This dose has already been shown to produce nephrotoxicity. The animals of group III received 80 mg/kg of gentamycin i.p., with cystone 5 ml/kg p.o., nearly for eight

Reagents (Supplied in the kit)

Reagent 1: Buffered substrate, pH 10.0

Reagent 2: Chromogen reagent

Reagent 3: Phenol standard, 10 mg%

days. The animals of group IV received 80 mg/kg of gentamycin i.p., nearly for eight days in addition to this, they also received 250 mg/kg of *Phyllanthus amarus* seeds methanolic extract orally. Group V animals were given 80 mg/kg b.w., of gentamycin i.p., for eight days in addition to this they also received 300 mg/kg of *Phyllanthus amarus* seeds aqueous extract orally.

This was started three days prior to the gentamycin injections and continues with eight days gentamycin treatment. At the end, animals were sacrificed under mild ether anaesthesia and blood samples were collected and assessed.

Blood sample collection

Blood samples of the animals were collected from retro orbital plexus from the inner canthus of the eye under mild ether anaesthesia, using capillary tubes before the animals were sacrificed. The plasma was separated in a T8 electric centrifuge at 2000 rpm for 2 minutes assessment and then analysed the for hepatoprotective and nephroprotective biochemical parameters (Md. Liyakat *et al.*, 2012), using the standard procedures, prescribed in the enzymatic kits (MEDES LIMITED, U. K.).

Statistical analysis

Results were expressed as $\bar{x} \pm SE_{\bar{x}}$, (n = 6). Statistical analysis was performed with one-way-analysis of variance (ANOVA), followed by Turkey-Kramer multiple comparisons test by using

Graph Pad Instat software. p value less than 0.05 was considered to be statistically significant. * $p < 0.05$, ** < 0.01 and *** < 0.001 , when compared with control and toxicant group as applicable.

Results and Discussion

There was increased level of SGPT, observed in thioacetamide treated group (299.58 U/L), which was restored to 101.56 U/L by 250 mg/kg 70% methanolic extract of the seeds as that of 100mg/kg silymarin *i.e.*, 98.33 U/L. SGOT levels increased significantly in thioacetamide treated group *i.e.*, 403.35 U/L. 250 mg/kg 70% methanolic extract of the seeds reduced to 108.56 U/L, which was very near to 100 mg/kg silymarin effect of 106.13 U/L. In case of the total and direct bilirubin, 250 mg/kg 70% methanolic extract reduced the elevated levels of total and direct bilirubin levels by *i.e.*, from 2.38 mg/dl and 0.61 mg/dl to 1.03 mg/dl and 0.26 mg/dl, respectively. The effect of 250 mg/kg 70% methanolic extract had better than 100 mg/kg standard silymarin. There was no significant rise in total cholesterol and triglyceride levels in thioacetamide treated group. 250 mg/kg 70% methanolic extract was almost same as that of standard 100 mg/kg silymarin. There was an increase in ALP level, observed in thioacetamide treated group (468.73 IU/L). The level was restored to 149.70 IU/L by 250 mg/kg 70% methanolic extract of the seeds which was near to effect of 100 mg/kg silymarin *i.e.*, 145.45 IU/L. Increased biochemical parameters were restored significantly by 250 mg/kg 70% methanolic extract which shows that the seeds have hepatoprotective activity.

Gentamycin induced kidney damage, shown very high decrease of body weight in gentamicin treated animals (-10.31%). However, there was an equal decrease in body weights in animals treated with aqueous extract 250 mg/kg - 7.17%. 70% methanolic extract, showed decrease in body weight by -4.12%. When compared to standard cystone 5ml/kg *i.e.*, -1.14%. Blood urea nitrogen level increased in gentamicin treated group *i.e.*, 59.68 mg/dl. 250 mg/kg 70% methanolic and aqueous extracts of seeds restored the elevated level to 30.06 mg/dl and 47.16 mg/dl, respectively, with compared with the standard cystone 5 ml/kg *i.e.*, 28.01 mg/dl. Serum creatinine level also increased in gentamicin treated group to 1.74 mg/dl. However, 250 mg/kg 70% methanolic extract of seeds reduced the elevated level to 0.96 mg/dl with that of standard cystone 5 ml/kg to 0.95 mg/dl. Toxicant group have shown significant decrease in body weight, 70% methanolic extract of seeds was restored back to normal levels. Also the increased creatinine and urea was restored back to normal. Hence, the results show that the seeds possess hepatoprotective and nephroprotective activities.

Histopathological studies

Shown the protective effect of *Phyllanthus amarus seed* extracts and were further confirmed by histopathological examination of liver.

Normal group : Sections studied show structure of liver. Architecture is normal. Central vein, sinusoids and hepatocytes are normal.

Thioacetamide group : Sections showed marked necrosis and inflammatory cell infiltration in the centrilobular area, inflammatory cells were also observed in the portal triad. Development of fibrosis septae was observed between central veins and portal triad.

Silymarin group : Sections studied shows structure of liver. The architecture is maintained. There is mild congestion and portal triaditis and inflammatory cells in the centrilobular area.

Methanolic group : Sections studied showed greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein showing regeneration of hepatocytes.

Aqueous group : Sections studied features of mild necrosis with mild congestion and reduced necrosis area around central veins.

Many drugs or chemicals injure the liver and kidney. Hepatoprotective and nephroprotective activity of several herbal extracts, using different models has been reported by several researchers (Singanan *et al.*, 2007; Maldonado *et al.*, 2003 and Yaman and Balikei, 2010). It seems that the 70% methanolic extract could more protect the liver and kidney than aqueous extract against thioacetamide and gentamycin induced oxidative damage possibly by reducing the enzymatic levels and increasing the antioxidant defense mechanism in rats (Faremi *et al.* 2008).

Conclusion

Many antioxidants have been used to protect the organs from these free radical challenges. Keeping in view of its antioxidant and organ protective activities, the present research work is made by use of Wister rats to assess the influence of pre-treatment with extracts of *Phyllanthus amarus* seeds. Liver and kidney functions were assessed by collecting the blood samples from each group and evaluating the biochemical parameters. Histopathological studies were done by isolating the liver and kidney of all the groups.

Treatment with 70% ethanolic 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 thioacetamide induced hepatotoxicity in rats to near normal levels. Whereas 70% methanolic extract prevented the reduction in body weight and reduced the levels of total urea and serum creatinine in gentamicin induced nephrotoxicity.

Histopathological observation exhibited the improved hepatic and renal anatomy that is reversed the damage almost equal to standard drugs. Thus, 70% methanolic extract of *Phyllanthus amarus* seeds possess hepatoprotective and nephroprotective activity.

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

Treatment GROUPS	Biochemical parameters Mean \pm SE $_{\bar{x}}$						
	SGPT U/L	SGOT U/L	Total Bilirubin mg/dl	Direct Bilirubin Mg/dl	Total Cholesterol Mg/dl	ALP U/L	Triglyc erides mg/dl
Negative control (1ml vehicle)	75.78 \pm 2.818	87.38 \pm 6.121	0.93 \pm 0.047	0.28 \pm 0.023	112.74 \pm 3.585	129.68 \pm 5.013	176.94 \pm 3.667
Positive control (Thioacetamide) + Tween 80 (1:1) 100 mg/kg, s.c	299.58 \pm 10.722	403.35 \pm 8.667	2.38 \pm 0.116	0.61 \pm 0.070	190.54 \pm 11.128	469.35 \pm 7.218	215.87 \pm 10.264
Thioacetamide + Std (Silymarin) 100 mg/kg, s.c + 100 mg/kg, p.o	98.33 \pm 4.043***	106.13 \pm 6.927***	1.05 \pm 0.061***	0.29 \pm 0.030***	125.28 \pm 5.656***	146.62 \pm 7.408*	174.37 \pm 9.903**
Thioacetamide + 70% meth. extract 100 mg/kg, s.c + 250 mg/kg, p.o	134.67 \pm 1.069***	141.49 \pm 1.198**	1.19 \pm 0.060***	0.47 \pm 0.013	156.08 \pm 0.954**	188.78 \pm 1.108**	191.63 \pm 0.595
Thioacetamide + aqueous extract 100 mg/kg, s.c + 300 mg/kg, p.o	101.53 \pm 0.667***	108.56 \pm 1.134**	1.03 \pm 0.025***	0.26 \pm 0.016***	129.31 \pm 0.981**	149.70 \pm 0.905**	177.49 \pm 0.815*

Values are the $\bar{x} \pm SE_{\bar{x}}$ of six rats / treatment

Significance *p<0.05, **p<0.01 and ***p<0.001 compared to thioacetamide treatment

Table 3: Effect of 70% methanolic extract of *Phyllanthus amarus* seeds on gentamicin induced renal damage in rats

Treatment	Change in b.w (%)	Blood urea (mg/dl)	Serum creatinine (mg/dl)
Negative control(1ml vehicle)	5.44 \pm 0.583	27.348 \pm 2.946	0.93 \pm 0.065
Positive control Gentamycin + Tween 80 (1:1) 80 mg/kg, i.p	-10.31 \pm 1.03	59.68 \pm 4.205	1.74 \pm 0.082
Gentamycin + Standard (cystone) 80 mg/kg, i.p. + 5 ml/kg, p.o	-1.14 \pm 0.546***	28.01 \pm 1.782**	0.95 \pm 0.015**
Gentamycin + 70% meth. extract 80 mg/kg, i.p. + 250 mg/kg, p.o	-4.12 \pm 0.505***	30.06 \pm 0.782*	0.96 \pm 0.025*
Gentamycin + aqueous extract 80 mg/kg, i.p. + 300 mg/kg, p.o	-7.17 \pm 1.964*	47.16 \pm 0.884	1.18 \pm 0.075

Values are $\bar{x} \pm SE_{\bar{x}}$ of six rats / treatment

Significance **p<0.01 and ***p<0.001 (vs. Control). b.w. – Body weight

In the present study, test extract shown presence of polyphenolic compound and demonstrated antioxidant activity. The 70% methanolic extract 250 mg/kg of *Phyllanthus amarus* seeds have shown promising results as compared to other extracts. Therefore, the study concludes that the 70% methanolic extract of *Phyllanthus amarus* seeds have antioxidant and organ protective property.

The results obtained in this study indicate that *Phyllanthus amarus* seed possess hepatoprotective and nephroprotective activity. Methanolic extract effects were comparable to silymarin and cystone, and were highly significant, whereas aqueous extract were less potent compared standard drugs.

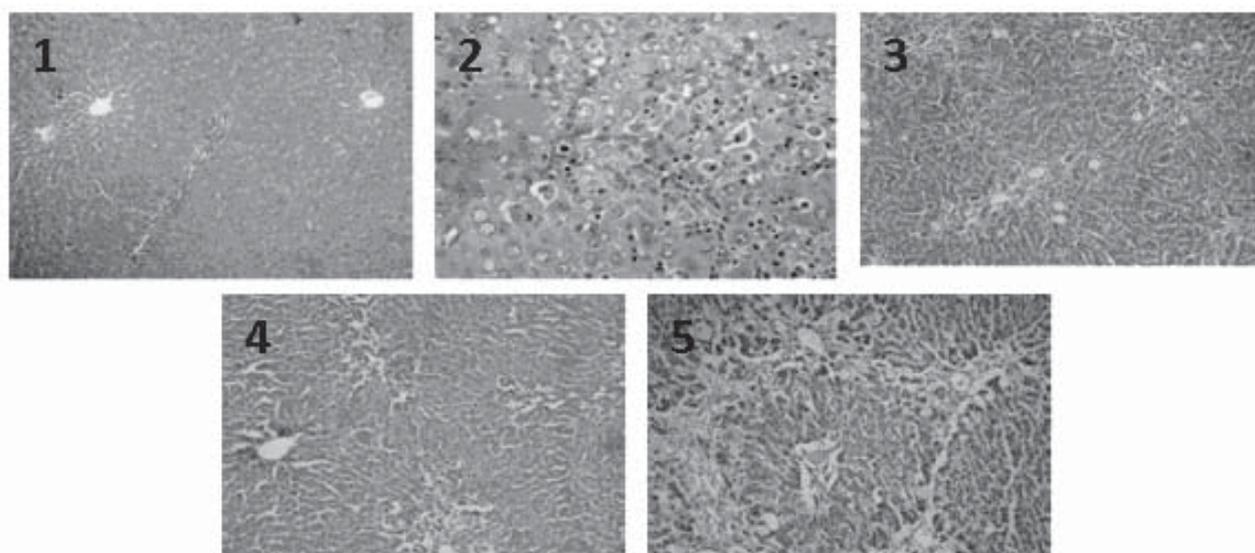


Figure 1: (1: NORMAL GROUP: Sections studied show structure of liver. Architecture is normal. Central vein, sinusoids and hepatocytes are normal. 2: THIOACETAMIDE GROUP: Sections showed marked necrosis and inflammatory cell infiltration in the centrizonal area, inflammatory cells were also observed in the portal triad. Development of fibrosis septae was observed between central veins and portal triad. 3: SILYMARIN GROUP: Sections studied shows structure of liver. The architecture is maintained. There is mild congestion and portal triaditis and inflammatory cells were observed in the centrizonal area. 4: METHONOLIC GROUP: Sections studied showed greater reduction of the necrosed area and sparse inflammatory cell infiltration around the central vein showing regeneration of hepatocytes. 5: AQUEOUS GROUP: Sections studied features of mild necrosis with mild congestion and reduced necrosis area around central veins).

Acknowledgment

I am thankful my Principal, Nizam Institute of Pharmacy and Research Centre, for his suggestions and helping me at each and every step of completion of this research work successfully.

References

- Ali, S.A.; Al-amin, T.H.; Mohamed, A.H. and Gameel, A.A. (2009). Hepatoprotective activity of aqueous and methanolic extracts of *Capparis deciduas* stems against carbon tetrachloride induced liver damage in rats. *J. Pharmacol. and Toxicol.*, **4**: 167-72.
- Best and Taylors (1984). *Physiological basis of medical practice*. 11th Ed. Williams and Wilkins. London, pp: 451-544.
- Chattopadhyay, R.R. (2003). Possible mechanism of hepatoprotective activity of *Azadirachta indica* leaf extract. Part II. *J. Ethnopharmacol.*, **89**: 217-219.
- Diener, W.; Mischke, U.; Kayser, D. and Schlede, E. (1995). The biometric evaluation of the OECD modified version of the acute toxicity class method (oral). *Arch. Toxicol.*, **69**: 729-734.
- Faremi, T.; Susu, S.M.; Fafunso, M.A. and Obioha, U.E. (2008). Hepatoprotective potentials of *Phyllanthus amarus* against ethanol induced oxidative stress in rats. *Food Chem. Toxicol.*, **46**: 2658-2664.
- Ghosh, M.N. (1985). Toxicity studies. *In: Fundamentals of Experimental Pharmacology*. Scientific book agency. Calcutta, pp:153-158.
- Maldonadu, P.D.; Barrera, D.; Rivero, I.; Mata, R.; Copos, O.N. and Pando, R.H. (2003). Antioxidant S-allcystein prevents gentamycin-induced oxidative stress and renal damage. *Bio. Med.*, **35**: 317-24.
- Md. Liyakat, A.; Ashwini, J.; Paramjyothi, S.; Sanaullah, S. and Santosh N.K. (2012). Antiulcer and hepatoprotective effects of *Semicarpus anacardium* Linn. seed extract. *Annals of Phytomedicine*, **1**(1): 105-109.
- Obianime, A.W. and Uche, F.I. (2008). The phytochemical screening and the effects methanolic extract of *Phyllanthus amarus* leaf on the biochemical parameters of male Guinea pigs. *J. Appl. Sci. Environ. Manage.*, **12**: 73-77.
- Pearson, P.G. and Wienkers, L.C. (2009). *Handbook of Drug Metabolism*, 2nd Ed. Informa. Healthcare, pp: 64-69.
- Reitmnn, S. and Frankel, S. (1957). *American Journal of Clinical Pathology*, **34**: 131.
- Reitmnn, S. and Frankel, S. (1957). *American Journal of Clinical Pathology*, **28**: 56.
- Shahani, S. (1999). Evaluation of hepatoprotective efficacy of APCLA polyherbal formulation *in vivo* in rats. *Ind. Drugs*, **36**: 628.

Singanan, V.; Singanan, M. and Begam Haseena (2007). The hepatoprotective effect of bael leaves in alcohol induced liver injury in albino rats. *Intl. J. Sci. Technol.*, **2**: 83-92.

Thyagarajan, S.P.; Jayaram, S.; Gopalakrishnan, V.; Hari, R.; Jeyakumar, P. and Sripathi, M.S. (2002). Herbal medicines for liver diseases in India. *J. Gastroenterol. Hepatol.*, **17**: 370-376.

Turner, M.A. (1965). *Screening methods in Pharmacology*. Academic Press, New York, pp: 26.

Vijay Kumar, K.; Naidu, M.U.R.; Anwar, A. and Ratnakar, K.S. (2000). Protocol protects against gentamycin induced nephrotoxicity in rats. *Ind. J. Pharmacol.*, **32**: 108-113.

William, G.M.; Elizabeth, K. and Weinberger, H.J. (1971). Isolation of long term cell culture of epithelial like cells from rat liver. *Exp. Cell. Res.*, **69**: 106-112.

Yaman, I. and Balikci, E. (2010). Protective effects of *Nigella sativa* against gentamycin-induced nephrotoxicity in rats. *Exp. Toxicol. Pathol.*, **62**(2): 183-90.