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Pharmacological Evaluation of *P. Amarus* Seeds and *L. Aspera Leaves* For Its Hepatoprotective and Nephroprotective Activities

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ABSTRACT

Hepatic and Renal systems are involved in the metabolism and excretion of various xenobiotics, environmental pollutants etc. Hence they are highly prone to attack by oxidative stress and generation of excessive concentration of free radicals. This results in tissue necrosis and damage of these organ systems. Keeping in view of its antioxidant and organ protective activities, the present research work is made by use of Albino Rats (Wister) to assess the influence of pre-treatment with methanolic extract of *Phyllanthus amarus* seeds and ethanolic extract of *Leucas aspera* leaves in simvastatin induced hepatotoxicity, where as in cisplatin induced nephrotoxicity. Liver and Kidney functions were assessed by collecting the blood samples from each group & evaluating the biochemical parameters. Histopathological studies were done by isolating the liver and kidney of all the groups. Treatment with methanolic and ethanolic extracts has brought back the elevated levels of serum glutamate pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), alkaline phosphatase (ALP), total & direct bilirubin, serum cholesterol and serum triglycerides in simvastatin induced hepatotoxic rats to near normal levels. Whereas methanolic extract, ethanolic extract and aqueous extract prevented the reduction in body weight and reduced the levels of total urea and serum creatinine in cisplatin induced nephrotoxicity. Histopathological observation exhibited the improved hepatic and renal anatomy that is reversed the damage. Thus methanolic extract of *Phyllanthus amarus* seeds and ethanolic extract of *Leucas aspera* leaves possess hepatoprotective and nephroprotective activities, comparatively *Leucas aspera* leaves shows better results, this may be due to presence of phenolic compounds like flavonoids steroids and tannins known to possess antioxidant activity.

Keywords: *Phyllanthus amarus, Leucas aspera, Simvastatin-induced, Cisplatin-induced*

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INTRODUCTION

Liver is an important organ actively involved in many metabolic functions and is the frequent target for a number of toxicants [1]. Hepatic damage is associated with distortion of these metabolic functions [2]. Liver disease is still a worldwide health problem. Unfortunately, conventional or synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects [3]. 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 synthetic agents severe undesirable side effects 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 [4]. Therefore an effective formulation has to be developed using indigenous medicinal plants, with proper pharmacological experiments and clinical trials.

Phyllanthus amarus

The plant *Phyllanthus amarus* is widely distributed in all tropical regions of the planet. Paleobotanical studies have not found the exact geographic origin of this plant. This plant may be indigenous to the tropical Americas [5-7]. *Phyllanthus amarus* is a common pantropical weed that grows well in moist, shady and sunny places [5]. The fruit is a three lobed capsule extending from the cup and commonly the long stalk pendant [8]. The name 'Phyllanthus' means "leaf and flower" because the flower, as well as the fruit, seems to become one with the leaf [5].

Phyllanthus amarus Plant

Phyllanthus amarus Fruits

Phyllanthus amarus is an erect annual herb, 10 to 50 cm high, with smooth cylindrical stem 1.5 to 2 mm thick and deciduous horizontal branchlets 4 to 12 cm long and about 0.5 cm thick. The seed capsules on stalks are 1 to 2 mm long, round, smooth, 2 mm wide, with 6 seeds. When the fruits burst open the seeds are hurled away. Seeds are triangular (like an orange segment), light brown, 1 mm long, with 5 to 6 ribs on the back [6].

Leucas aspera

Leucas aspera is distributed throughout India from the Himalayas down to Ceylon. The plant is an annual, branched, herb erecting to a height of 15-60 cm with stout and hispid acutely quadrangular stem and branches. Leaves are sub-sessile or shortly petiolate, linear or linearly lanceolate, obtuse, pubescent up to 8.0 cm long and 1.25 cm broad, with entire or crenate margin; petiole 2.5-6 mm long, TS of leaf passing through the midrib is broadly convex on the lower side and slightly grooved or flat on the upper side, a centrally located conjoint and collateral meristele associated with a parenchymatous pericycle layer on lower side, collenchymatous tissue underneath both the epidermis; dorsiventral lamina epidermis covered with thick cuticle, traversed with stomata, bears simple and glandular trichomes of the same type as found on stem, 1 to 2 layered palisade tissue occupying the major area of the section and spongy parenchyma.

Leucas aspera Fruit

Leucas aspera Leaves

The plant is used traditionally as an antipyretic and insecticide. Medicinally, it has been proven to possess various pharmacological activities like antifungal, antioxidant, antimicrobial, antinociceptive and cytotoxic activity. Further, studies reveal the presence of various phytochemical constituents mainly triterpenoids, oleanolic acid, ursolic acid and b-sitosterol, nicotine, sterols, glucoside, diterpenes, phenolic compounds (4-(24-hydroxy-1-oxo-5-n-propyltetracosanyl)-phenol). These studies reveal that *L. aspera* is a source of medicinally active compounds and have various Pharmacological effects; hence, this drug encourage finding its new therapeutic uses. *L. aspera* plant is used traditionally as an antipyretic and insecticide. Flowers are valued as stimulant, expectorant, aperients, diaphoretic, insecticide and emmenagogue. Leaves are considered useful in chronic rheumatism, psoriasis and other chronic skin eruptions. Bruised leaves are applied locally in snake bites.

A number of medicinal plants are used in traditional system of medicinal for the management of liver disorders. Nature has given us a large number of medicinal plants, some of which are yet to be explored and validated for their medicinal value. The 21st century has seen a paradigm shift toward therapeutic evaluation of herbal products in liver diseases, carefully synergizing the strengths of traditional medicine with the modern concept of evidence based medical evaluation, standardization and randomised placebo controlled clinical trials to support clinical efficacy. Several herbs are known to possess antioxidant properties and may be useful as liver protective agents [9].

The herbs containing antioxidant principles are reported to be highly effective in preventing or curing the liver toxicities due to above mentioned challenges. In the present study, the herb *Phyllanthus amarus* containing polyphenolic compounds is selected to assess hepatoprotective activity [10].

MATERIALS AND METHODS

Plant material

The seeds of *Phyllanthus amarus* and leaves of *Leucas aspera* were collected from local gardens of Tirupathi. The plant was identified and authenticated by Madava Chetty, Department of Botony, Sri Venkateshwara University, Tirupathi, A. P, India.

Preparation of Extracts

The crushed and dried seeds of *Phyllanthus amarus* was extracted successively with methanol by soxhlet extraction and concentrated by rotary vacuum [11]. The leaves of *Leucas aspera* was extracted by cold maceration process for aqueous extraction [12]. 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 to analyze the reparative activity of liver injury due to simvastatin treated rats.

Experimental Animals

The Wister rats weighing 180-200g were collected from Mahaveer Agencies. The animals were allowed to acclimatize for 7 days in new environment before the experiment. The rats had free access to standard pellet chow and water *ad libitum* throughout the experiment. They were housed in a cage of six animals per cage.

Acute Oral Toxicity Studies

The acute oral toxicity study is determined according to the guidelines of Organization for Economic Co-operation & Development (OECD) following the up & down method (OECD guideline No. 423). Based on the method, a limit test was performed to categorize the toxicity class of the compound and then main test was performed on three female rats to estimate the exact LD50. The animals were fasted overnight with free access to water, weighed and a single dose of the test substance was administered. Animals were observed individually during first 30 min, periodically during 48 h with special attention given during first 4 h (short-term toxicity) and daily, thereafter for total of 14 days (short-term toxicity). LD50 was found to be greater than 2500 mg/kg, in limit test. The test substance could be classified in the hazard classification as Class 5 - 2000 mg/kg

<LD50 <5000 mg/kg in the globally harmonized system (GSH). LD50 of test drug was found to be 2500mg/kg from main test [13].

Hepatotoxins and Nephrotoxins

It is emphasized that hepatotoxins that cause acute hepatitis should have close resemblance with the viral hepatitis, clinically, biochemically and histopathogically. Certain drugs are also responsible for many hepatic diseases, such as chronic hepatitis, fatty liver, cirrhosis and certain vascular lesions of liver. In many instances drug induced hepatitis is distinguishable from viral hepatitis, unlike other hepatotoxins, simvastatin is one of the most powerful hepatoxin in terms of severity of injury it causes toxic necrosis leading to biochemical changes having clinical features similar to those of acute viral hepatitis [14], liver injury was produced by administration of simvastatin mixed with tween 80. Animals were given single doses simvastatin 20 mg/kg, p.o., per day throughout the experimental setup. Control animals received an equal volume of tween 80.

The term renal failure primarily denotes failure of the excretory function of kidney, leading to retention of nitrogenous waste products of metabolism in the blood. In addition, there is failure in the regulation of fluid electrolyte balance along with endocrine dysfunction. The renal failure is fundamentally categorized into acute and chronic renal failure [15,16]. Acute renal failure (ARF) refers to the sudden and usually reversible loss of renal function which develops over a period of days of weeks. There are many causes of acute renal failure which could be either failure pre-renal (55%), renal (40%) or post renal (5%). Among the renal causes of acute renal failure, acute tubular necrosis is more common accounting for 85% of incidence. Acute tubular necrosis occurs either due to ischemia or toxins. Kidney injury was produced by administration of cisplatin. Animals were given dose of 6 mg/kg, i.p, per day through out the experimental setup. Control animals received an equal volume of saline.

Evaluation of hepatoprotective activity [17]

***Methanolic extract of Phyllanthus amarus* Schum & Thonn seeds**

Animals are divided into 5 groups, each comprising 6 rats.

Group I : Control group (1ml tween 80 p.o)
Group II : Simvastatin treated (20mg/kg, p.o)
Group III : Simvastatin (20mg/kg, p.o) +Silymarin (20mg/kg, p.o)
Group IV : Simvastatin (20mg/kg, p.o) + *Phyllanthus amarus seeds* (150mg/kg, p.o)
Group V : Simvastatin (20mg/kg, p.o) + *Phyllanthus amarus seeds* (300mg/kg, p.o)

Animals were divided into five different groups, each having 6 rats and treated accordingly. Group 1: rats received a normal standard diet for 30 days; Group 2: Rats received SMT (20 mg/kg p.o for 30 days); Group 3: Rats received SMT along with silymarin (20mg/kg p.o for 30 days); Group 4: Rats received SMT along with *Phyllanthus amarus seeds* extract (150mg/kg, p.o for 30 days); Group5: Rats received SMT along with *Phyllanthus amarus seeds* extract (300mg/kg, p.o for 30 days).

On the 31st day, all the animals were sacrificed by mild ether anaesthesia.

***Ethanolic extract of Leucas aspera* Spreng leaves**

Animals are divided into 5 groups, each comprising 6 rats.

Group I : Control group (1ml tween 80 p.o)
Group II : Simvastatin treated (20mg/kg, p.o)
Group III : Simvastatin (20mg/kg, p.o) +Silymarin (20mg/kg, p.o)
Group IV : Simvastatin (20mg/kg, p.o) + *Leucas aspera* leaves (150mg/kg, p.o)
Group V : Simvastatin (20mg/kg, p.o) + *Leucas aspera* leaves (300mg/kg, p.o)

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Hepatoprotection Standard drug (Silymarin 35mg /5ml)

Silymarin, an Ayurvedic formulation of Micro drug company, Bangalore is used mainly in the treatment of liver disorders. Silymarin was administered in a dose of 100 mg/kg p.o., into rats.

Evaluation of nephroprotective activity

***Methanolic extract of Phyllanthus amarus* Schum & Thonn seeds [18]**

Six groups of six rats in each were used in this model. They were weighed individually on first and last day of treatment**.** The study carried out for 7 days

Group I : Control group (1ml tween 80 p.o)
Group II : Cisplatin treated (6mg/kg, i.p)
Group III : Cisplatin treated (6mg/kg, i.p) + Cystone 5 ml/kg p.o
Group IV : Cisplatin treated (6mg/kg,i.p) + *Phyllanthus amarus seeds* 150mg/kg, p.o
Group V : Cisplatin treated (6mg/kg, i.p) + *Phyllanthus amarus seeds* 300mg/kg

On 2nd day, after 30 min of tween 80, 5 ml/kg cystone, 150 mg/kg, 300 mg/kg methanolic extract of *Phyllanthus amarus* seeds were administered to Group-I, III, IV, and V respectively, received cisplatin 6 mg/kg i.p., Group- II, III, IV, and V respectively were administered once a day on days 1, 4, 5 & 6 and twice a day on days 2 and 3.

***Ethanolic extract of Leucas aspera* Spreng leaves**

Six groups of six rats in each were used in this model. They were weighed individually on first and last day of treatment**.** The study carried out for 7 days

Group I : Control group (1ml tween 80 p.o)
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Group IV : Cisplatin treated (6mg/kg,i.p) + *Leucas aspera* leaves 150mg/kg, p.o
Group V : Cisplatin treated (6mg/kg, i.p) + *Leucas aspera* leaves 300mg/kg, p.o

On 2nd day, after 30 min of tween 80, 5 ml/kg cystone, 150 mg/kg, 300 mg/kg ethanolic extract of *Leucas aspera* leaves were administered to Group-I, III, IV, and V respectively, received cisplatin 6 mg/kg i.p., Group- II, III, IV, and V respectively were administered once a day on days 1, 4, 5 & 6 and twice a day on days 2 and 3.

Nephroprotection Standard drug (Cystone 50mg /5ml)

Cystone, an Ayurvedic formulation of Himalaya drug company, Bangalore is used mainly in the treatment of kidney disorders. Cystone was administered in a dose of 5 ml/kg p.o., into rats.

RESULTS

Effect of methanolic extract of *Phyllanthus amarus* seeds on biochemical markers in Simvastatin induced hepatotoxicity

There was increased level of SGPT, SGOT and ALP observed in simvastatin treated groups (312.42 U/L, 318.41 U/L and 235.86 IU/L respectively). The extract showed a dose dependent effect. SGPT levels was

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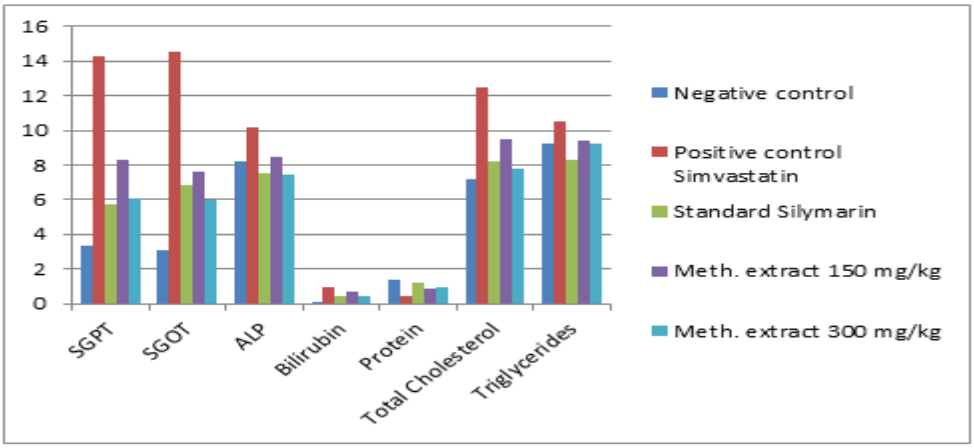
restored to 77.84 U/L, SGOT levels was restored to 72.69 U/L and ALP levels was restored to 97.44 U/L by 300mg/kg methanolic extract of the seeds which was near to the effect of 100mg/kg silymarin i.e. 65.39 U/L, 71.21 U/L and 95.68 U/L respectively. In case of the total bilirubin and protein, a dose dependent effect of the extract was observed. 300mg/kg methanolic extract reduced the elevated levels of total bilirubin i.e. from 4.892 mg/dl to 1.20 mg/dl with compared to standard 1.54 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 simvastatin treated group. Dose dependent effect was observed with the methanolic extract and of 300 mg/kg. Methanolic extract was comparable with 100 mg/kg silymarin.

Effect of methanolic extract of *Phyllanthus amarus* seeds on biochemical markers in Simvastatin induced hepatotoxicity



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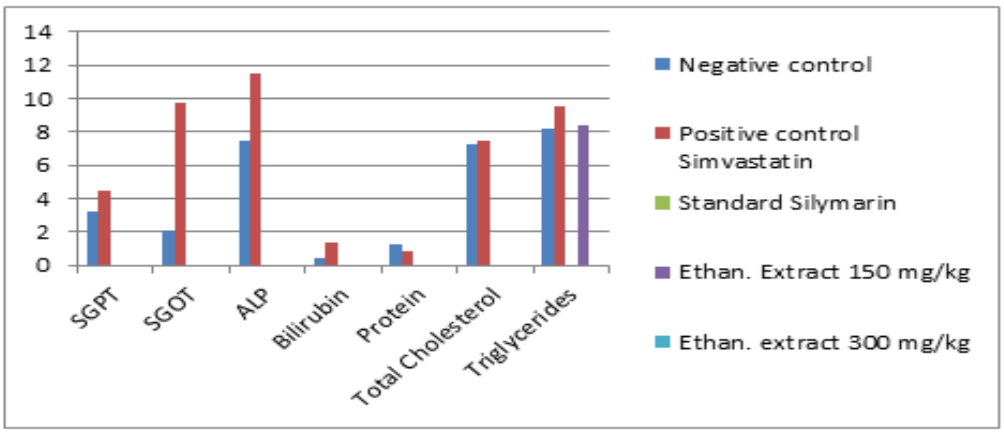
Effect of ethanolic extract of *Leucas aspera* leaves on biochemical markers in Simvastatin induced hepatotoxicity



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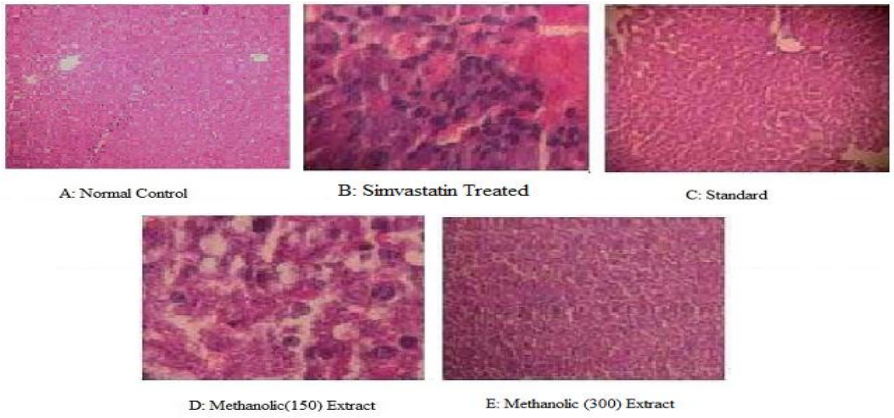
Histopathological studies

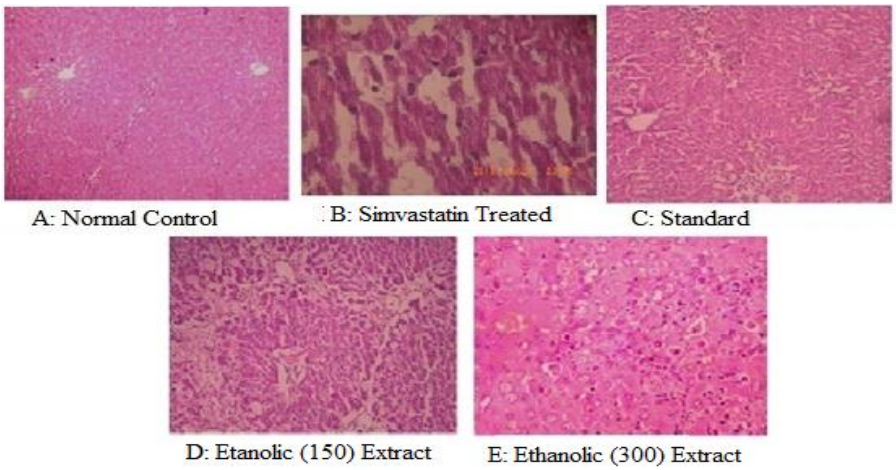
All the animals were sacrificed by mild ether anaesthesia. The blood samples were collected from retro orbital plexus for evaluating the serum biochemical parameters and liver was dissected out, blotted off blood, washed with saline and stored in 10% formalin and preceded for histopathology to evaluate the details of liver and kidney's architecture in each group microscopically.

Histopathological studies of *Phyllanthus amarus* seeds on Simvastatin induced hepatotoxicity



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 Simvastatin 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 300 mg/kg methanolic extract group the hepatic architecture was maintained. Areas of kupffer cells proliferation and sinusoids appeared to be normal.

Histopathological studies of *Leucas aspera* leaves on Simvastatin induced hepatotoxicity



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 Simvastatin 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 ethanolic 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 300 mg/kg ethanolic extract group the hepatic architecture was maintained. Regenerated tubular epithelium and healing of necrotic changes.

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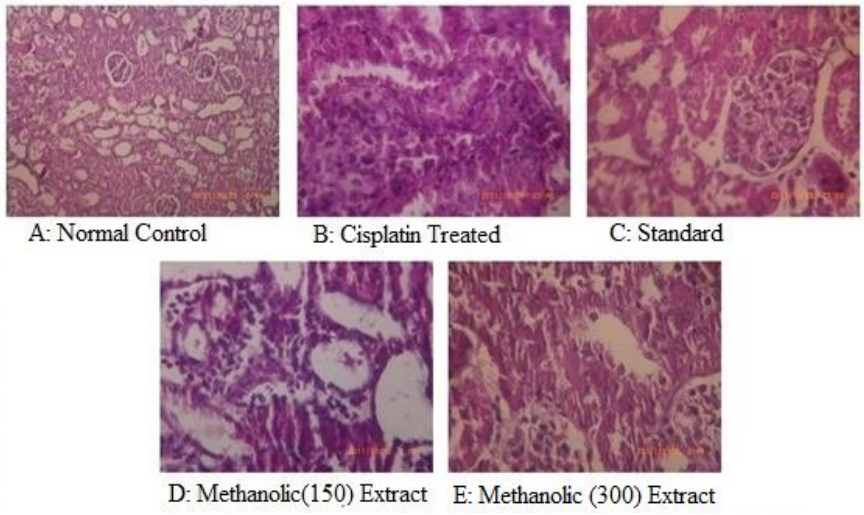
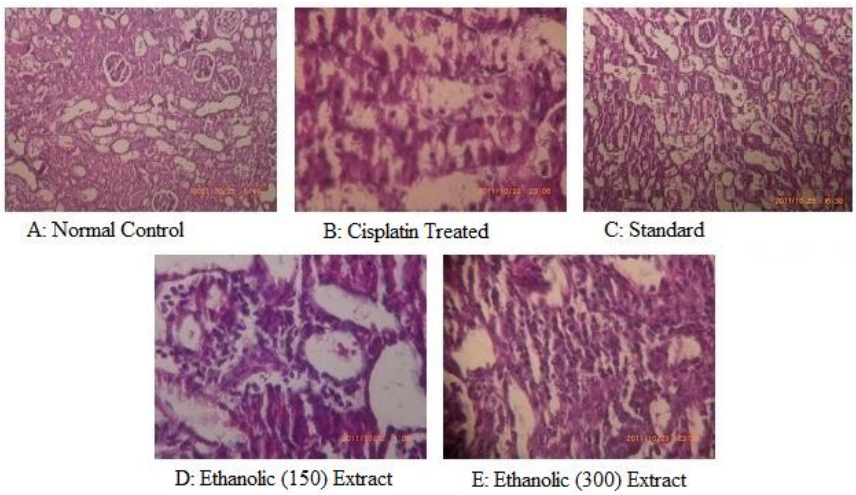
Histopathological studies in methanolic extract of *Phyllanthus amarus* on cisplatin induced nephrotoxicity



Group A: Normal control group showed structure of kidney with normal glomeruli, proximal and distal tubules with normal interstitium and blood vessels.
Group B: (Massive total necrosis). Cisplatin treated group showed structure of kidney with glomerular congestion. Interstitium showed infiltration with inflammatory cells, tubular necrosis, peritubular necrosis and presence of casts.
Group C: (Interstitial Inflammation). In the case of 5 ml/kg cystone treated group showed structure of kidney with normal glomeruli, proximal and tubules with interstitium showing few lymphocytes.
Group D: (Interstitial Nephritis). In the case of 150 mg/kg methanolic extract group showed structure of kidney with normal glomeruli, extensive cloudy swelling with focal nephritis and interstitial inflammation.
Group E: (Necrosis with Interstitial Inflammation). In the case of 300 mg/kg methanolic extract group showed structure of kidney with normal glomeruli and cloudy change in tubules with moderate to small foci of necrosis and interstitial inflammation.

Histopathological studies in ethanolic extract of *Leucas aspera* leaves on cisplatin induced nephrotoxicity



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Group C: (Interstitial Inflammation). In the case of 5 ml/kg cystone treated group showed structure of kidney with normal glomeruli, proximal and tubules with interstitium showing few lymphocytes.
Group D: (Scanty Inflammation). In the case of 150 mg/kg ethanolic extract group showed structure of kidney with normal glomeruli, extensive cloudy swelling with focal scanty inflammation between tubules.
Group E: (Residual Interstitial Nephritis). In the case of 300 mg/kg ethanolic extract group showed structure of kidney with normal glomeruli, proximal and distal tubules with interstitium showing scant lymphatic infiltration.

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