Vol 6| Issue 1| 2016 | 32-40.

e-ISSN 2248 – 9142
print-ISSN 2248 – 9134

International Journal of
Current Pharmaceutical & Clinical Research

www.ijcpcr.com

EVALUATION OF PROTECTIVE EFFECT OF *PHYLLANTHUS VIRGATUS* AGAINST CARBON TETRACHLORIDE AND PARACETAMOL INDUCED HEPATOTOXICITY IN RATS

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ABSTRACT

The hepatoprotective activity of methanolic extract of the whole plant *Phyllanthus virgatus* was studied on Wistar albino rats. Carbon tetrachloride (CCl_4) and paracetamol were used to induce hepatotoxicity. Acute oral toxicity study was performed as per OECD-423 guidelines. The hepatoprotective activity of the plant extract was determined by assessing serum parameters (SGOT, SGPT, total bilirubin and total protein) and by examination of hepatic tissues. Silymarin was used as the standard drug. The biochemical results demonstrated that both the doses of methanolic extract of *Phyllanthus virgatus* decreased CCl_4 and PCM induced elevated enzyme levels, and increased the levels of total protein, indicating the protection of structural integrity of hepatocytic cell membrane and the regeneration of damaged liver cells. Histopathological studies showed that the plant extract decreased periportal inflammation and infiltration of inflammatory cells particularly lymphocytes in liver. These results suggest that whole plant of *Phyllanthus virgatus* possesses significant hepatoprotective properties.

Key words: *Phyllanthus virgatus*, Carbon tetrachloride, Paracetamol, Silymarin, Hepatoprotective activity.

INTRODUCTION

Liver is the key organ regulating homeostasis in the body. It is involved with almost all the biochemical pathways related to growth, fight against disease, nutrient supply, energy production and reproduction. Because of its unique metabolism and relationship to the gastrointestinal tract, the liver is an important target of the toxicity of drugs, xenobiotics and oxidative stress [1]. In the absence of reliable liver protection drugs in modern medicine, a large number of medicinal preparations are recommended for the treatment of liver disorders, resulting in various adverse effects. Thus, there is a severe necessity to carry out research on herbal hepatoprotective drugs to minimize adverse effects.

Oxidative stress has been identified to be the major cause of hepatotoxicity which provides that plants with anti-oxidant chemical constituents would be useful in this regard. In review of literature, it was found that the plant '*Phyllanthus virgatus*' contains tannins and flavonoids as the major chemical constituents [2] and thus has the antioxidant potential. Keeping this in view, the plant *Phyllanthus virgatus* is expected to have a protective role in liver toxicities induced by different chemicals and environmental challenges.

It is also well documented that carbon tetrachloride (CCl_4) and paracetamol triggers hepatic and renal changes in animals and man [3]. Their mechanism of action is also very well illustrated by several authors and hence the same have been opted to induce hepatotoxicity [4]. Therefore, the present study is aimed to evaluate the hepatoprotective activity of the plant extract by assessing serum parameters (SGOT, SGPT, total bilirubin and total protein) and to perform histopathological examination of hepatic tissues.

MATERIALS AND METHODS

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Animals

Wistar albino rats of either sex were used for the study. The animals were housed in groups of six and maintained under standard conditions and fed with standard rat diet and purified drinking water ad libitum. All the procedures described in the study were approved by the Institutional Animal Ethical Committee (IAEC) of Albino Research & Training Institute, Hyderabad and with the permission from Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA/IAEC/EXP/25/50/2013/EXP-09), Ministry of Social Justice and Empowerment, Government of India.

Plant material

Phyllantus virgatus whole plant was collected from Tirupati, Andhra Pradesh. The plant was identified and authenticated by Dr. K. Madhava Chetty, Assistant Professor, Department of Botany, Sri Venkateswara University, Trupati, Andhra Pradesh. After authentication, plant was cleaned and milled into coarse powder by a mechanical grinder.

Preparation of methanolic extract of *Phyllantus virgatus* whole plant

Phyllantus virgatus whole plant (1kg) was extracted with 95% methanol using a soxhlet apparatus. The methanolic extract was filtered and concentrated by distillation process. A brownish green color residue was obtained (yield 6.79% w/w) and was kept in desiccator. This methanolic extract of *Phyllantus virgatus* whole plant was used for further experiment.

Acute toxicity studies

Acute oral toxicity study was performed as per OECD-423 guidelines. Wistar albino rats (n = 6) of either sex were selected by random sampling technique. The animals were kept fasting for overnight provided only with water, after which the extract was administered orally at the dose level of 1000 mg/kg of body weight and observed for 24 hours. If mortality was observed in 2 out of 3 animals, then the dose administered was assigned as toxic dose. If mortality was observed in 1 animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for further higher dose such as 2000 mg/kg and 3000 mg/kg of body weight.

EXPERIMENTAL DESIGN

Hepatoprotective activity

A. Pharmacological studies

Experimental animals were divided randomly into five groups each consisting of six animals.

Method 1: Carbon Tetrachloride (CCl_4) induced hepatoxicity

In the CCl_4 induced liver toxicity model, CCl_4 (0.5 ml/kg i.p.) was administered daily for 7 days to all the animals except Group-1 [5].

Group-1: normal control administered with normal saline (1ml/kg, p.o)

Group-2: toxin control - CCl_4 in Olive Oil (1:1 ratio) (1ml/kg, i.p)

Group-3: standard - drug silymarin (50mg/kg, p.o) and CCl_4 (0.5ml/kg, i.p)

Group-4: CCl_4 induced hepatotoxic rats treated with methanolic extract of *Phyllanthus virgatus* at a dose of 100mg/kg, p.o.

Group-5: CCl_4 induced hepatotoxic rats treated with methanolic extract of *Phyllanthus virgatus* at a dose of 200mg/kg, p.o.

The animals were sacrificed 24 h after last treatment under light anesthetic ether. Blood from each rat was withdrawn by retro orbital plexus under ether anesthesia for biochemical investigation i. e. SGOT, SGPT, total bilirubin and total protein estimation. Blood was allowed to coagulate at 37^0C for 30 min and the serum was separated by centrifugation at 2500 rpm for 15 min. The liver of one animal from each group was removed and processed immediately for histopathological investigation.

Method 2: Paracetamol induced hepatotoxicity

In the paracetamol induced liver toxicity model, paracetamol (1g/kg, p.o) diluted with sucrose solution will be administered on 7th day to all animals except Group-1 [6].

Group-1: normal control administered with normal saline (1ml/kg, p.o)

Group-2: toxin control - paracetamol in 50 % sucrose solution (1g/kg, p.o)

Group-3: standard - drug silymarin (50mg/kg p.o) and paracetamol (1g/kg, p.o)

Group-4: paracetamol induced hepatotoxic rats treated with methanolic extract of *Phyllanthus virgatus* at a dose of 100mg/kg, p.o.

Group-5: paracetamol induced hepatotoxic rats treated with methanolic extract of *Phyllanthus virgatus* at a dose of 200mg/kg, p.o.

The animals will be sacrificed 24hr after last treatment under light anesthetic ether. Blood from each rat will be withdrawn by retro orbital plexus under ether anesthesia for biochemical investigation i. e. SGOT, SGPT, total bilirubin and total protein estimation. Blood will be allowed to coagulate at 37^0C and 30 min and the serum will be separated by centrifugation at 2500 rpm for 15 min. The liver of one animal from each group will be removed and processed immediately.

BIO-CHEMICAL STUDIES

The biochemical parameters (SGPT, SGOT, TB and TP) were estimated as per the standard procedure prescribed by the manufacturer's instruction manual

provided in the kit (Coral clinical systems, Verna Goa, India) using Autoanalyser (ARTOS).

Estimation of SGPT (ALT)
Principle

Alanine amino transferase (ALT) catalyses the transfer of amino group from L-Alanine to 2-oxo glutarate with the formation of pyruvate and L-glutamate [7]. The pyruvate so formed is allowed to react with NADH to produce L-lactate. The rate of this reaction is monitored by an indicator reaction coupled with LDL in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGPT activity.

Procedure

Pipette	Sample (µl)
Working reagent	1000

Mix well and aspirate.

Estimation of SGOT (AST)
Principle

Aspartate transaminase (AST) catalyses the transfer of amino group from L- Aspartate to 2-oxo glutarate with the formation of oxaloacetate and L-glutamate. The rate of this reaction is monitored by an indicator reaction coupled with malate dehydragenase (MDL) in which the oxaloacetate formed is converted to malate ion in the presence of NADH (nicotinamide adenine dinucleotide). The oxidation of NADH in this reaction is measured as a decreasing in the absorbance of NADH at 340 nm, which is proportional to SGOT activity.

Procedure

Pipette	Sample (µl)
Working reagent	1000
Sample	100

Mix well and aspirate.

Estimation of total bilirubin
Principle

Bilirubin reacts with diazotized sulphanilic acid in acidic medium to form a pink coloured azobilirubin with absorbance directly proportional to bilirubin concentration. Direct bilirubin, being water soluble directly react in acidic medium. However, indirect and unconjugated bilirubin is solubilised using a surfactant and then it reacts similar to direct bilirubin.

Procedure

	Blank(µl)	Standard	Test
Working	500	500	500
Distilled	25	-----	-----
Standard	-----	25	------
Sample	-----	-----	25

Mix well. Incubate for 5 minutes at 37^{o}C temperature for Total bilirubin and direct bilirubin. Read absorbance at 546/630 nm against Reagent blank.

Estimation of total proteins
Principle

The peptide bond of proteins reacts with Cu^{+2} ions in alkaline solution to from a blue violet complex (Biuret reaction), each copper ion complexing with 5 or 6 peptide bonds. Tarterate is added as stabilizer while iodine is used to prevent auto reduction of alkaline copper complex. The color formed is proportional to the protein concentration and is measured at 546 nm.

Procedure

	Blank(µl)	Standard	Test
Working	1000	1000	1000
Distilled	20	-----	-----
Standard	-----	20	------
Sample	-----	-----	20

Incubate for 10 min. at 37^{0}c. Read absorbance of standard and each sample at 546 nm against reagent blank.

Calculations for percent recovery
Carbon tetrachloride

Percent reduction of tissue parameters SGOT, SGPT and Total bilirubin (TB) were calculated in the following manner:

$$\text{Percent reduction} = \frac{\left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in CCl 4 group}\end{array}\right] - \left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in treated group}\end{array}\right]}{\left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in CCl 4 group}\end{array}\right] - \left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in vehicle control group}\end{array}\right]} \times 100$$

Percent protection of Total protein (TP) was calculated in the following manner

$$\textbf{Percent protection} = \frac{\left[\begin{array}{l}TP\\ \text{levels in treated group}\end{array}\right] - \left[\begin{array}{l}TP\\ \text{levels in CCl 4 group}\end{array}\right]}{\left[\begin{array}{l}TP\\ \text{levels in vehicle control group}\end{array}\right] - \left[\begin{array}{l}TP\\ \text{levels in CCl 4 group}\end{array}\right]} \times 100$$

Paracetamol

Percent reduction of tissue parameters SGOT, SGPT and Total bilirubin (TB) were calculated in the following manner

$$\text{Percent reduction} = \frac{\left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in PCMgroup}\end{array}\right] - \left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in treated group}\end{array}\right]}{\left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in PCMgroup}\end{array}\right] - \left[\begin{array}{l}\text{SGOT / SGPT /}\\ \text{TB}\\ \text{levels in vehicle control group}\end{array}\right]} \times 100$$

Percent protection of Total protein (TP) was calculated in the following manner

$$\text{Percent protection} = \frac{\left[\begin{array}{l}\text{TP}\\ \text{levels in treated group}\end{array}\right] - \left[\begin{array}{l}\text{TP}\\ \text{levels in PCM group}\end{array}\right]}{\left[\begin{array}{l}\text{TP}\\ \text{levels in vehicle control group}\end{array}\right] - \left[\begin{array}{l}\text{TP}\\ \text{levels in PCM group}\end{array}\right]} \times 100$$

Statistical analysis

Results were expressed as Mean±S.E.M. Statistical analysis was performed by one way ANOVA followed by Tukey test with the help of Graph Pad Prism 5.0 software.

RESULTS

Acute toxicity study

In the acute toxicity study, it was observed that none of the doses (i.e. 1000mg/kg, 2000mg/kg & 3000mg/kg of b.w) produced any lethality among the tested animals when administered as a single dose. The animals did not show any gross behavioural changes except for an increase in urination indicating the safe usage of the extract at a dose of 100 mg/kg and 200 mg/kg.

HEPATOPROTECTIVE ACTIVITY

Effect of methanolic extract of *P. virgatus* on serum biochemical parameters

Effect on serum glutamate oxaloacetate transaminases (SGOT)

There was a significant increase in the SGOT levels in the CCl_4 group and paracetamol group when compared to the vehicle control group. Silymarin treated group showed a significant reduction in the SGOT levels when compared to toxin CCl_4 group and toxin paracetamol group. The trend was same with that of plant extract treated groups suggesting the healing of damaged hepatocytes by our extract [Table 1 & 6 and fig. 1 & 5].

Effect on serum glutamate pyruvate transaminases (SGPT)

A significant elevation in the SGPT levels was observed in the CCl_4 group and paracetamol group when compared to the vehicle control group. The standard group showed a significant reduction in the SGPT levels when compared to toxin CCl_4 group and toxin paracetamol group. So was the case with that of plant extract treated groups indicating the regeneration of hepatocytes [Table 2 & 7 and fig. 2 & 6].

Effect on serum total bilirubin (TB)

The serum TB levels were found to rise significantly in case of CCl_4 group and paracetamol group when compared to vehicle control group. The standard group showed a significant reduction in the TB levels when compared to toxin CCl_4 group and toxin paracetamol group. So was the case with that of plant extract treated groups implying the stability of biliary function **[**Table 3 & 8 and fig. 3 & 7].

Effect on serum total proteins (TP)

There was a significant reduction in the serum TP in the CCl_4 group and paracetamol group when compared to the vehicle control group. Whereas a significant increase was observed in case of the standard and plant extract treated groups demonstrating the regeneration process of the liver [Table 4 & 9 and fig. 4 & 8].

CARBON TETRACHLORIDE

Table 1. Effect of methanolic extract of *Phyllanthus virgatus* on serum aspartate aminotransferase (AST or SGOT) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	43.48±0.09
Group 2	CCl_4 control	102.73±0.21***
Group 3	Standard (50 mg/kg, p.o)	48.25±0.14***
Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	68.19±0.10***
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	62.38±0.11**

Values are expressed as mean±S.E.M; n=6, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$ considered for significance, (ANOVA followed by tukey test)

Table 2. Effect methanolic extract of *Phyllanthus virgatus* on serum alanine aminotransferase (ALT or SGPT) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	72.79±0.215
Group 2	CCl_4 control	385.75±0.70***
Group 3	Standard (50 mg/kg, p.o)	57.24±0.23***

Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	60.51±0.22***
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	59.20±0.27***

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by tukey test).

Table 3. Effect of methanolic extract of *Phyllanthus virgatus* on serum total bilirubin (TB) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	0.26±0.04
Group 2	CCl_4 control	2.05±0.17***
Group 3	Standard (50 mg/kg, p.o)	0.30±0.009*
Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	0.35±0.07***
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	0.26±0.008***

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by tukey test)

Table 4. Effect of methanolic extract of *Phyllanthus virgatus* on serum total protein (TP) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	7.48±0.87
Group 2	CCl_4 control	2.16±0.01***
Group 3	Standard (50 mg/kg, p.o)	6.80±0.07***
Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	3.73±0.006***
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	6.15±0.02***

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by tukey test)

Table 5. Percent Recovery Table of Carbon tetrachloride

Treatment	Dose	% Reduction of Serum Biomarkers			%Protection of Serum Biomarkers
		SGOT	SGPT	Total Bilirubin	Total Protein
Methanolic extract of *Phyllanthus virgatus*	100 mg/kg, p.o	58	93	94	29
	200 mg/kg, p.o	68	95	96	75
Standard drug Silymarin	50 mg/kg, p.o	91	96	97	87

PARACETAMOL

Table 6. Effect of methanolic extract of *Phyllanthus virgatus* on serum aspartate aminotransferase (AST or SGOT) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	161.63±0.89
Group 2	PCM control	650.51±13.02***
Group 3	Standard (50 mg/kg, p.o)	191.51±3.30*
Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	236.45±2.17**
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	169.9±0.35***

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by tukey test).

Table 7. Effect of methanolic extract of *Phyllanthus virgatus* on serum alanine aminotransferase (ALT or SGPT) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	53.71 ±0.08
Group 2	PCM control	384.78±0.63***
Group 3	Standard (50 mg/kg, p.o)	67.85±0.52*
Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	116.5±0.69**
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	77.01±0.35***

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by tukey test)

Table 8. Effect of methanolic extract of *Phyllanthus virgatus* on serum total bilirubin (TB) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	0.27±0.004
Group 2	PCM control	1.69±0.09***

Group 3	Standard (50 mg/kg, p.o)	0.30±0.01***
Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	0.61±0.04*
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	0.44±0.02**

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by tukey test)

Table 9. Effect methanolic extract of *Phyllanthus virgatus* on serum total protein (TP) levels

Group	Treatment	Mean ± SEM
Group 1	Vehicle control	7.89 ± 0.31
Group 2	PCM control	5.02 ± 0.19***
Group 3	Standard (50 mg/kg, p.o)	5.79 ± 0.19*
Group 4	Methanolic extract of *P. virgatus* (100 mg/kg, p.o)	6.09 ± 0.10**
Group 5	Methanolic extract of *P. virgatus* (200 mg/kg, p.o)	6.90 ± 0.10***

Values are expressed as mean±S.E.M; n=6, *P<0.05, **P<0.01, ***P<0.001 considered for significance, (ANOVA followed by tukey test).

Table 10. Percent Recovery Table of Paracetamol

Treatment	Dose	% Reduction of Serum Biomarkers			%Protection of Serum Biomarkers
		SGOT	SGPT	Total Bilirubin	Total Protein
Methanolic extract of *Phyllanthus virgatus*	100 mg/kg, p.o	84	81	76	37
	200 mg/kg, p.o	98	92	88	65
Standard drug Silymarin	50 mg/kg, p.o	93	95	97	66

HISTOPATHOLOGICAL EXAMINATION:

Photomicrographs representing the effect of methanolic extract of *Phyllanthus virgatus* against Carbon tetrachloride induced hepatotoxicity

1.) Mild vacoular degeneration surrounding periportal region of liver.
2.) Periportal inflammation and infiltration of inflammatory cells particularly lymphocytes noticed in liver.
3.) Moderate vacoular and fatty degeneration observed in the surface of liver
4.) Hepatocytes appeared normal in periportal, portal and centri lobular region of liver. No inflammation and necrosis observed in the liver
5.) Hepatocytes appeared normal in periportal, portal and centri lobular region of liver

Photomicrographs representing the effect of methanolic extract of *Phyllanthus virgatus* against Paracetamol induced hepatotoxicity

1.) Periportal inflammation and infiltration of inflammatory cells noticed in liver
2.) Periportal region appeared normal
3.) Moderate foci of inflammation and infiltration of lymphocytes noticed in periportal region of liver
4.) Moderate sinusoidal hemorrhages in centri lobular region of liver

Fig 1. Effect of *P.virgatus* on SGOT levels in CCl_4 induced hepatoxicity

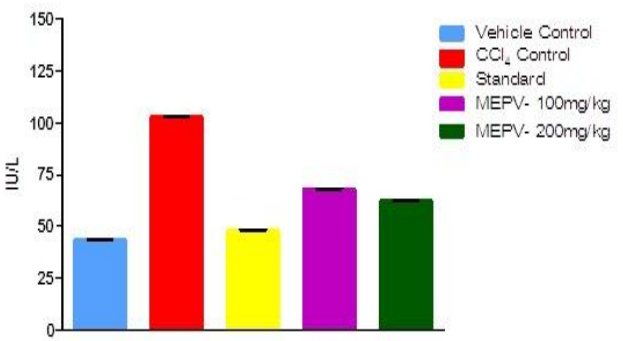


Fig 2. Effect of *P.virgatus* on SGPT levels in CCl_4 induced hepatoxicity

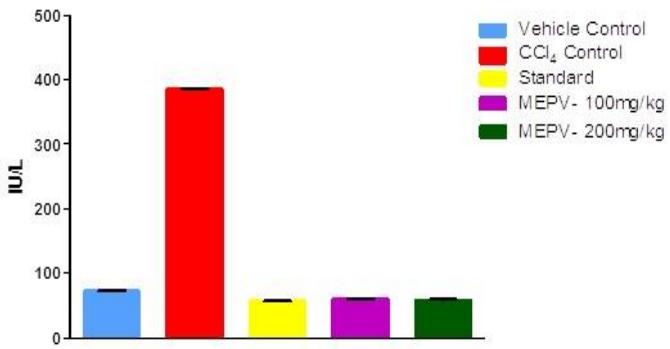


Fig 3. Effect of *P.virgatus* on total bilirubin levels in CCl_4 induced hepatoxicity

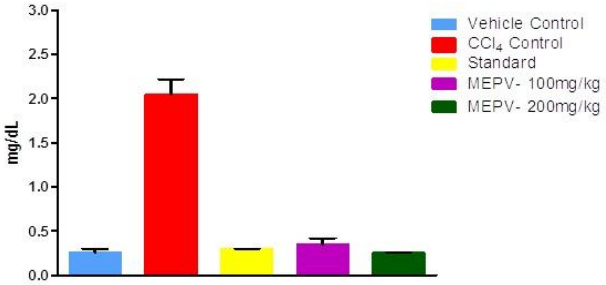


Fig 4. Effect of *P.virgatus* on total protein levels in CCl_4 induced hepatoxicity

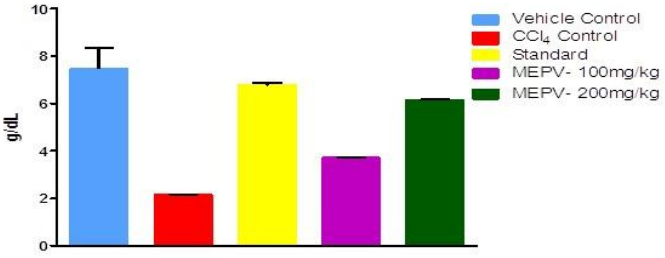


Fig 5. Effect of *P.virgatus* on SGOT levels in PCM induced hepatoxicity

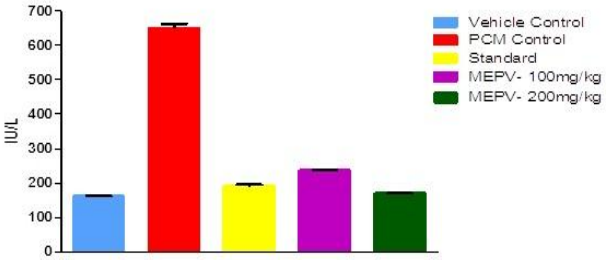


Fig 6. Effect of *P.virgatus* on SGPT levels in PCM induced hepatoxicity

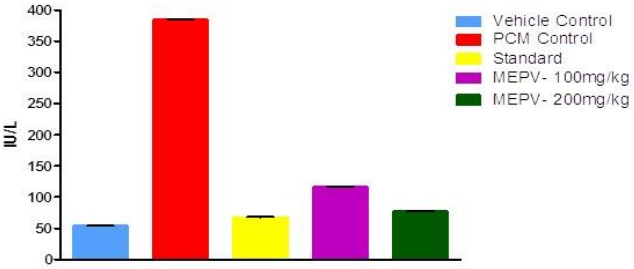


Fig 7. Effect of *P.virgatus* on total bilirubin levels in PCM induced hepatoxicity

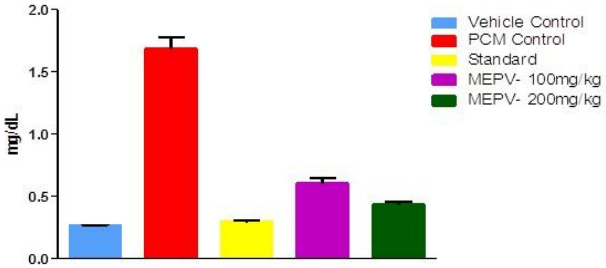


Fig 8. Effect of *P.virgatus* on total protein levels in PCM induced hepatoxicity

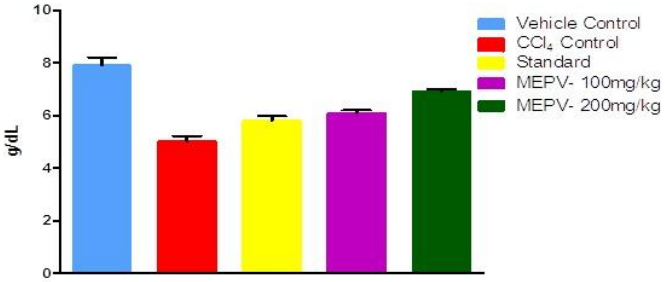


Fig 9. Vehicle control

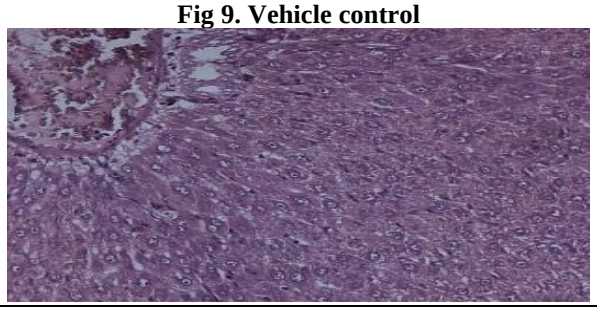


Fig 10. CCL_4 control

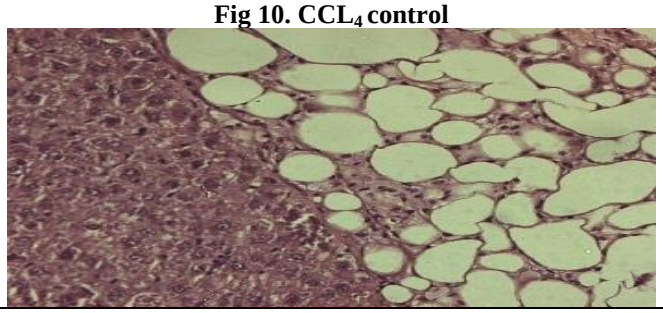


Fig. 11. Standard

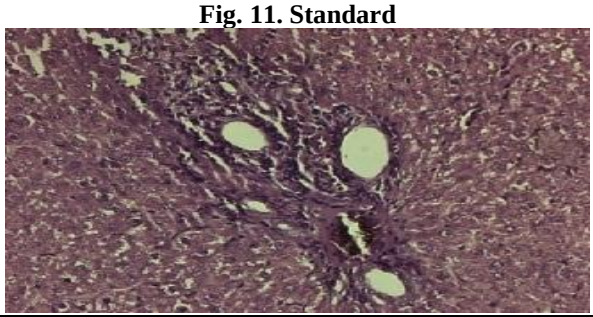


Fig 12. Met. Ext. of *P. virgatus* extract at a dose of 100mg/kg

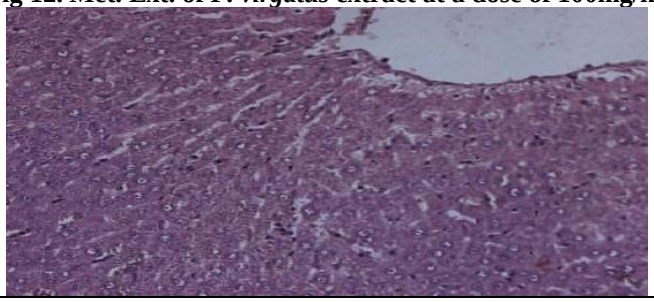


Fig 13. Met. Ext. of *P. virgatus* extract at a dose of 200mg/kg

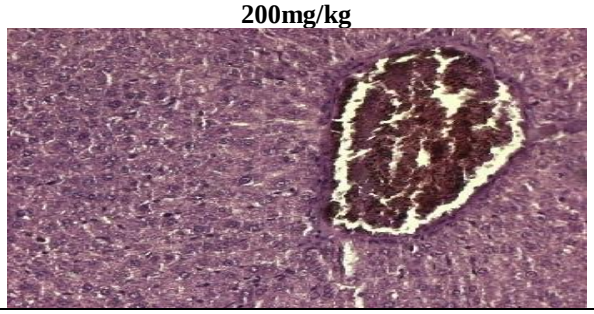


Fig 14. Paracetamol control

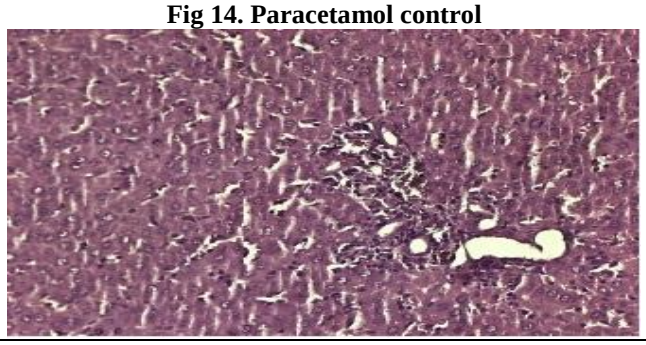


Fig 15. Standard

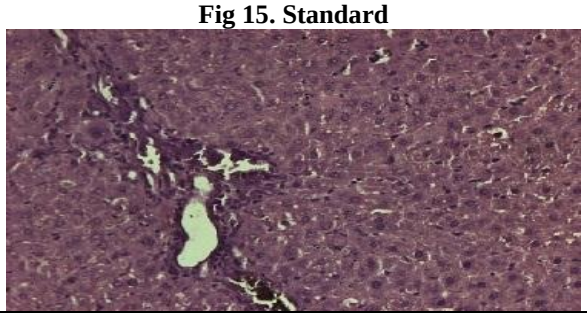


Fig 16. Met. Ext. of *P. virgatus* extract at a dose of 100mg/kg

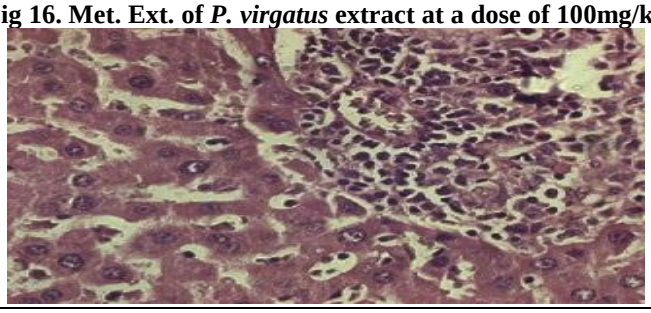
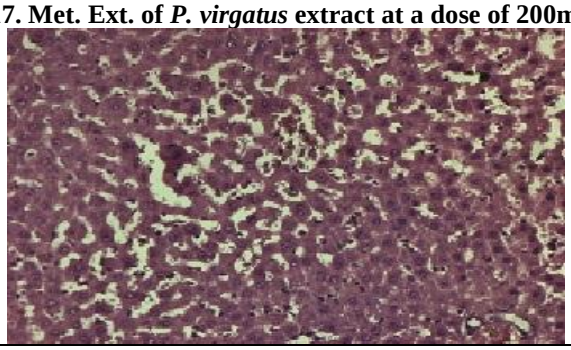


Fig 17. Met. Ext. of *P. virgatus* extract at a dose of 200mg/kg



DISCUSSION

Liver plays a major role in detoxification and excretion of many endogenous and exogenous compounds, any injury or impairment of its function may lead to several implications on one's health. It is therefore necessary to search herbal drugs for the treatment of liver diseases. The use of rats as experimental animals for hepatoprotective activity is mainly because of the structural homology of rat CYP 450 enzymes with that of humans [8].

Carbon tetrachloride is a routinely used hepatotoxin for experimental study of liver diseases.

Administration of CCl_4 causes acute liver damage that mimics natural causes. It mediates changes in liver function that ultimately leads to destruction of hepatocellular membrane leading to liver necrosis. The analgesic paracetamol causes a potentially fatal, hepatic centrilobular necrosis when taken in overdose. Paracetamol is metabolically activated by cytochrome P450 enzymes to a reactive metabolite that depletes glutathione (GSH) and covalently binds to protein. Formation of paracetamol protein adducts produces hepatocellular death. Necrosis or membrane damage produced by either CCl_4 or paracetamol releases enzymes into circulation which can be measured in the serum.

Amino transferases are liver specific enzymes that catalyse the interconversion of amino acids and α-keto acids by the transfer of amino groups. These include aspartate aminotransferase (AST or SGOT) and alanine aminotransferase (ALT or SGPT). These enzymes are normally predominantly contained within liver cells and to a lesser degree in the muscle cells. If the liver is injured or damaged, the liver cells spill these enzymes into the blood, raising the AST and ALT enzyme blood levels and signaling liver disease [9]. Plant extract could significantly lower the elevated amino transferase levels when compared to both carbon tetrachloride group and paracetamol group. This indicates healing of hepatic parenchyma and the regeneration of hepatocytes.

Bilirubin is produced by the normal breakdown of pigment-containing proteins, especially hemoglobin from senescent red blood cells and myoglobin from muscle breakdown. Bilirubin released from such sources, tightly

albumin bound, is delivered to the liver, where it is efficiently extracted and conjugated by hepatic glucuronidation and sulfation. Conjugated bilirubin is rapidly excreted into bile and removed from the body through the gut. An elevated level of conjugated serum bilirubin implies liver disease. There was a remarkable reduction in the bilirubin levels by both the doses of methanolic extract of *Phyllanthus virgatus* when compared to both carbon tetrachloride group and paracetamol group, implying its potential as hepatoprotective agent.

The liver is also known to play a significant role in the serum protein synthesis [10]. The reduction in the total protein (TP) is attributed to the initial damage produced and localised in the endoplasmic reticulum which results in the loss of CYP 450 leading to its functional failure with a decrease in protein synthesis and accumulation of triglycerides leading to fatty liver. Both the doses of methanolic extract of *Phyllanthus virgatus* considerably enhanced the synthesis of total protein which may be by accelerating the regeneration process and protecting the liver cells. The increased levels of total protein in serum are indicative of the hepatoprotective activity.

The extent of hepatic damage is assessed by histological evaluation along with the levels of various biochemical parameters in circulation. The animals in both the carbon tetrachloride and paracetamol groups showed severe hepatotoxicity evidenced by profound steatosis, centrilobular necrosis, ballooning degeneration, nodal formation and fibrosis as compared to the normal hepatic architecture of the vehicle control animals. In plant extract treated animals, hepatocytes appeared normal in periportal, portal and centri lobular regions of liver. There was no inflammation and necrosis in plant extract treated liver.

In summary, this study suggests that oral administration of *Phyllantus virgatus* significantly ameliorates carbon tetrachloride and paracetamol induced hepatotoxicity in rats. The plant extract may possibly protect the liver by free radical scavenging activity and thus preventing peroxidation of lipids of the endoplasmic reticulum.

CONCLUSION

The efficacy of any hepatoprotective drug is dependent on its capacity of either reducing the harmful effect or restoring the normal hepatic physiology that has been disturbed by a hepatotoxin. The biochemical results demonstrated that both the doses of the plant extract (i.e. 100 mg/kg, p.o. and 200 mg/kg, p.o) prevented CCl_4 induced and paracetamol induced hepatotoxicity in rats. The methanolic extract of *P.virgatus* decreased the elevated SGOT, SGPT and total bilirubin levels and increased the reduced total protein levels. The effect was produced in a dose dependent manner. Histopathological studies showed that the plant extract decreased periportal inflammation and infiltration of inflammatory cells particularly lymphocytes in liver. From all these findings we can conclude that plant *Phyllantus virgatus* has good hepatoprotective activity as evidenced by biochemical parameters and histopathological examination.

ACKNOWLEDGEMENT

Nil

CONFLICT OF INTEREST

No interest

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