Vol 5| Issue 2| 2015 | 78-85.

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

International Journal of
Current Pharmaceutical & Clinical Research

www.ijcpcr.com

EVALUATION OF HEPATOPROTECTIVE AND ANTIOXIDANT ACTIVITY OF *Phyllanthus virgatus* AGAINST EXPERIMENTAL INDUCED HEPATOTOXICITY IN MALE WISTAR RATS

Paritala Deepthi*

CMR College of Pharmacy, Kandlakoya, Medchal Road, Hyderabad-501401, Telangana, India.

ABSTRACT

Evaluation of hepatoprotective activity and antioxidant activity of *Phyllanthus virgatus* against experimental induced hepatotoxicity in male wistar rats. The *in-vivo/ in vitro* antioxidant activity of whole plant of methanolic extract of *phyllanthus virgatus* was estimated of GSH and lipid peroxidation against CCl_4 and paracetamol hepatotoxicity in Wistar rats. Hepatoprotective activity of methanolic extract of *phyllanthus virgatus* was tested against carbon tetrachloride and paracetamol induced hepatotoxicity in Wistar rats. The degree of protection was determined by measuring levels of biochemical parameters like SGOT, SGPT, Total Bilirubin, ALP, Total cholesterol,. The histopathological studies were also carried out. Silymarin was used as the standard drug for comparison. This study aimed on Evaluating Hepatoprotective activity of *Phyllanthus virgatus* showed marked decrease in hepatotoxic effect of methanol which was evident by study of biochemical parameters and showed significant *in-vivo/in vitro* antioxidant in different models. Treatment of animal with methanolic extract of phyllanthus virgatus whole plant significantly ($p<0.01$) decreased the level of SGOT which is an indicative of hepatoprotective activity. Also brought down the level of SGPT significantly ($p<0.01$ in 200 mg/kg b.w and $p<0.01$) in 400mg/kg b.w doses) other then these parameters and levels of ALP, total cholesterol, also were indicative of hepatoprotective property of the plant extract. These results suggest that whole plant of *phyllanthus virgatus* possess potential antioxidant and hepatoprotective activities.

Key words: *phyllanthu virgatus*, Carbon tetrachloride, Paracetamol, Glutathione, Lipid peroxidation.

INTRODUCTION

Medicinal plants play a key role in the human health care. About 80% of the world population relies on the use of traditional medicine which is predominantly based on plant materials. The traditional medicine refers to a broad range of ancient natural health care practices including folk/tribal practices as well as Ayurveda, Siddha and Unani. These medical practices originated from time immemorial and developed gradually, to a large extent, by relying or based on practical experiences without significant references to modern scientific principles [1]. Herbal drugs constitute a major part of therapeutics in all the traditional systems of medicine which shows popular therapeutic diversity.

phyllanthus virgatus is which abundantly grown & used as important medicinal plant belonging to *Family phyllanthaceae.* The common name of this plant is seed under leaf, virgate leaf flower and the *Synonyms is P. simplex Retz.* The plant is useful in itch, abscess and gonorrhoea; used for measles and diseases in Khagrachari. Juice of the leaves is used in eye diseases. The fresh leaves, bruished and mixed with butter and milk is used as a wash to cure itch in children. Fresh leaves, flowers and fruit with cumin seed and sugar of equal parts made into an electuary, which is used for the cure of gonorrhoea. Root is useful in mammary abscesses.

However much of its medicinal importance is

Corresponding Author :- **Paritala Deepthi** Email:- paritaladeepthi@gmail.com

not assessed. By literature survey it is found that the leaves of the plant contain main secondary metabolites such as Luteolin, quercetin and β-sitosterol. It also contains phenols, flavonoids, alkaloids and tannins. This plant contain flavonoids hence we have planned to study its antioxidant and hepatoprotective property. Free radicals cause organ toxicities which are well reported. Therefore, there is a possibility that the antioxidants may have a protective role. Keeping this in view it was thought that the plant *phyllanhus virgatus* which is abundantly grown & used as medicinal plant may have a protective role in organ toxicities induced by different chemicals and environmental challenges [2]. An attempt is made during this research work to evaluate the aqueous and 70% methanolic extracts of whole plant of *phyllanthus virgatus* for *in-vivo* antioxidant activity. Alteration in the antioxidant status following ulceration indicates that free radicals seem to be associated with the pylorus ligation and ethanol induced ulceration in rats.Aspirin-induced ulceration is mediated through tissue damaging free radicals, which are produced from the conversion of hydroperoxyl to hydroxyl fatty acids, which leads to cell destruction. The hydroperoxyl fatty acids are generated from the degeneration of mast cells and generalized lipid peroxidation accompanying cell damage [3]. Therefore in the present study it is planned to assess the antioxidant and organ protective property of this plant. Hence from all this it is thought that the present study is highly justifiable and more needful.

MATERIALS AND METHODOLOY

Paracetamol also known as acetaminophen widely used as analgesic and antipyretic & Silymarin was used as standardized extract of milk thistle seeds containing a mixture of flavonolignans.

Collection of Plant

Phyllanthus vargatus whole plant was identified and authenticated by Dr. K. Madhava Chetty, Department of Botany, Sri Venkateswara University (Tirupati). The whole plant were shade dried at room temperature and pulverized.

Preparation of the Extract

The whole plant of *phyllanthus virgatus* were collected and shade dried under room temperature for 7 days to prevent the loss of active phyto constituents. The whole plant were subjected to size reduction to a coarse powdered using a mechanical grinder. The powdered material was soaked in 500ml of methanol in a conical flask. This was covered, shaken every 30 min. for 6hrs, allowed to stand for about 72hrs.The solution was subsequently shaken and filtered using what man filter paper. The filtrate was evaporated to dryness using a rotary evaporator. Hence the powdered material was extracted by maceration process.

Experimental Animal

Wistar rats of either sex and weighing 200 to 220 gm were housed in groups of 6 per cage under controlled light (12:12 light : dark cycle) and temperature (25+\-2^0c) environment and behavioral assessment was conducted during the light cycle. Food and water was provided. All procedures were carried out under strict compliance with ethical principles and guidelines of the animal ethical committee constituted. (CPCSEA)

Acute (Oral) Toxicity Study (Fixed Dose Procedure) Method

Acute toxicity studies for, Methanolic extracts of Phyllanthus vargatus whole plant belonging to the family 'phyllanthaceae' were conducted as per OECD using Wistar rats. Each animal was administered Methanolic extracts solution of the extract by oral route. The test procedur eminimizes the number of animals required to estimate the oral acute toxicity of achemical and in addition estimation of LD_{50}, confidence intervals. The test also allows the observation of signs of toxicity and can also used to identify chemicals that are likely to have low toxicity.

Principle of the Fixed Dose Procedure

The fixed dose procedure is method for assessing acute oral toxicity that involve the identification of a dose level that cause evidence of non-lethal toxicity (termed evident toxicity) rather than a dose level that cause lethality. Evident toxicity is a term describing clear signs of toxicity following administration of test substance, such that an increase to the next highest fixed dose would result in the development of severe toxic signs and probably mortality.

Treatment Schedule

Experimental rats were divided into 5 groups. Each group consists of 6 rats

Carbon tetrachloride (CCl_4) -Induced Hepatotoxicity

The animals were divided in to five groups of six animals.

Group 1 served as normal control.

Group 2 served as toxic control and received Ccl_4 (1ml/kg) daily once for 7 days.

Group 3 treated with standard drug (silymarin- 50mg/kg) and followed by Ccl_4 (1ml/kg) daily once for 7 days.

Group 4 served as test 1 and received Ccl_4 daily (1ml/kg) for 7 days then treated daily 7 days with plant extract (200 mg/kg).

Group 5 served as test 2 and received Ccl_4 daily (1ml/kg) for 7days then treated daily 7 days with plant extract (400mg/kg). After completion of treatment blood was

collected serum was seperated and used for biochemical parameters [4].

Collection of Blood Samples

All the animals were sacrificed on 7 th day under light ether anesthesia. The blood samples were collected separately in sterilized dry centrifuge tubes by puncture retro-orbital plexes and allowed to coagulate for 30 min at 37°C. The clear serum was separated at 2500rpm (Microcentrifuge) for 10min and subjected to biochemical investigation viz..,serum glutamic oxaloacetate transe aminase (SGOT), serum glutamic Pyruvate transe aminase (SGPT), Alkaline phosphatase (ALP) and Total Bilirubin (TB).Total Cholesterol

Paracetmol- Induced Hepatotoxicity

Total of 30 animal s divided in to 5 groups given orally the treatment for 7 days for standard,Test-1 Test-2 without inducing agent for 7 days

Group 1 Served as Normal control ,

Group 2 served as disease control PCM (1gm/kg) induce on 7^{th} day,

Group-3 Sillymarin (50mg/kg) given as a Standard drug for 7days and PCM was given on 7^{th} day,

Group-4 - Test 1: induce with extract for 7 days (200mg/kg) and paracetmol (1gm/kg) on 7^{th} day,

Group-5 - Test 2 treatment was given for 7 days with extract (400mg/kg) and paracetmol (1gm/kg) on 7^{th} day

Collection of Blood Samples

After 24 h of the last treatment, blood was collected from retro-orbital plexus, allowed to clot for 1 h at room temperature and serum was separated by centrifugation at 2 500 rpm at 30 °C for 15 min. The serum was then collected and analyzed for various biochemical parameters.

ANTIOXIDANT ACTIVITY

***In-vitro* Antioxidant Activity**

An attempt is made to assess the influence of pre-treatment with methanolic extract of *phyllanthus virgatus* whole plant on the levels of Glutathione and lipid peroxidation *in-vitro* in both CCl_4 and paracetamol induced hepatotoxicity.

a) Glutathione (GSH) and lipid peroxidation estimation in CCl_4 induced hepatotoxicity in Wistar rats of either sex.

b) Glutathione (GSH) and lipid peroxidation estimation in paracetamol induced hepatotoxicity in Wistar rats of either sex.

Estimation of Glutathione and Lipid peroxidation

Glutathione is present in all type of living cells. Tissue such as mammalian liver normally contains high levels of reduced Glutathione. It has been suggested that GSH protects thiol groups in protein from oxidation, functions as an intracellular redox buffer and serves as a reservoir of cysteine The role of GSH in determining the extent of liver damage has been demonstrated in experiments where the hepatic concentration of GSH is altered by toxin treatments. Depletion of GSH contents has been reported to potentiate hepatic necrosis and covalent binding of toxic metabolites to cellular macromolecules. Lipid peroxidation is accepted to be one of the principal cause of CCl_4 induced liver injury and is mediated by the production of free radical derivatives of CCl_4.

Estimation of GSH

Tissue samples were homogenized in ice cold Trichloroacetic acid (1 gm tissue plus 10 ml 10% TCA) in an ultra turrax homogenizer. Glutathione measurement was performed using a modification of the Ellamn procedure [5]. Briefly, after centrifugation at 3000 rpm for 10 minutes, 0.5 ml supernatant was added to 2 ml of 0.3 M disodium hydrogen phosphate solution. A 0.2 ml solution of dithiobis nitrobenzoate (0.4 mg/ml in 1% sodium citrate) was added and the absorbance at 412 nm was measured immediately after mixing. Percentage increase in D is directly proportional to the increase in the levels of Glutathione. Hence, % increase in OD is calculated. The results are compiled in Table 8

Estimation of lipid peroxidation

The degree of lipid peroxide formation was assayed by monitoring thiobarbituric reactive substances formation. Stock solution of TCA-TBA-HCl reagent: 15% w/v trichloroacetic acid; 0.375% thiobarbituric acid; 0.25N hydrochloric acid. This solution may be mildly heated to assists in the dissolution of the thiobarbituric acid.

Combine 1 ml of biological sample (0.1-0.2 mg of membrane protein) with 2 ml of TCA-TBA-HCL and mix thoroughly. The solution is heated for 15 min in a boiling waer bath. 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 results are compiled in Table 9 [6].

Estimation of SGPT (ALT)

Principal

Alanine aminotransferase (ALT) catalyser the transaminonation of L-Alanine and α-Ketoglutarate to form Pyruvate and L-Glutamate. In subsequent reaction, Lactate Dehydrogenase (LD) reduces Pyruvate to Lactate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide [reduced] (NADH) to Nicotinamide Adenine Dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm.

LD rapidly and cmpletely reduces endogenous Sample Pyruvate during the initial incubation period, so that it does not interfere with the assay [7].

L-Alanine + α-Ketoglutarate ⟶ < ALT > Pyruvate + L-Glutamate

Pyruvate + NADH + H^+ ⟶ < LD > L-Lactate + NAD

Estimation of SGOT (AST)
Principal

Aspartate aminotransferase (AST) catalyses the transmination of L-Aspartate and α-Ketoglutarate to form L-Glutamate and Oxaloacetate. In subsequent reaction, Malate Dehydrogenase (MDH) reduces Oxaloacetate to Malate with simultaneous oxidation of Nicotinamide Adenine Dinucleotide [reduced] (NADH) to Nicotinamide Adenine Dinucleotide (NAD). The rate of oxidation of NADH is measured kinetically by monitoring the decrease in absorbance at 340 nm and directly proportional to AST activity in the sample. LD is added to enzyme system to prevent endogenous Pyruvate interference, which is normally present in the Serum.

L- Aspartate + α-Ketoglutarate ⟶ < AST > Oxaloacetate + L-Glutamate

Oxaloacetate + NADH + H^+ < MDH > L-Malate + NAD

Estimation of Serum ALP
Principal

At pH 10.3 Alkaline Phosphatase (ALP) catalysesthe hydrolysis of colourless p-Nitrophenyl Phosphate (pNPP) to yellow coloured p-Nitrophenol and Phosphate. Change in absorbance due to yelloew colour formation is measured kinetically at 405 nm and proportional to ALP activity in the sample.

p- Nitro-phenylphosphate + H_2O < ALP > P-Nitrophenol + Phosphate ⟶

Procedure For ALT, AST & ALP

Pipette into tube marked	Test
Serum/Plasma	20µL
Working AST Reagent	1000µL

Mix well and aspirate immediately for measurement

Programme the analyser as per assay parameters. Blank the analyser with purified water. Read absorbance after 60 seconds. Repeat reading after every 30 seconds i.e. upto 120 seconds at 405 nm wavelength. Determine the mean absorbance change per minute (ΔA/minute).

Calculation of Serum ALT, AST & ALP

ALT, AST & ALP activity (IU/L) = ΔA/minute × Kinetic factor

Where, ΔA/minute = Change in absorbance per minute.

Kinetic factor (K) = 2712.

Estimation of Total bilirubin
Principle

Bilirubin reacts with diazotized sulfanilic acid in acidic medium to form pink colored azobilirubin with absorbance directly proportional to Bilirubin concentration. Direct Bilirubin, being water soluble directly reacts in acidic medium. However indirect or uncongugated Bilirubin is solubilised using a surfactant and then it react similar to Direct Bilirubin. Mix well, incubated for 5 minutes at 37ºC for Total and Direct Bilirubin. Read absorbance at 546/630 nm against Reagent blank.

Calculation with factor

Total Bilirubin (mg/dl) = Abs. of Test/Abs. of Standard × Factor (21).

Estimation of Serum Total Cholesterol followed by CHOD-PAP and PEG-CHOD-PAP, End Point Assay with Clearing Factor respectively
Principle for Serum Total Cholesterol

Cholesterol esters are hydrolysed by Cholesterol Esterase (CE) to give free Cholesterol and fatty acids. In subsequent reaction, Cholesterol Oxidase (CHOD) oxidases the 3-OH group of free Cholesterol to liberate Cholesterol-4-en-3-one and hydrogen peroxide. In presence of Peroxidase (POD), Hydrogen Peroxide couples with 4-Aminoantipyrine (4-AAP) and Phenol to produce Red Quinoneimine dye. Absorbance of coloured dye is measured at 505 nm and is proportional to amount of Total Cholesterol concentration in the sample.

Cholesterol esters <CE > Cholesterol + Fatty acids

Cholesterol+O_2<CHOD> Cholest-4-en-3one + H_2O_2

2 H_2O_2 + Phenol + 4-AAP<POD> Quinoneimine dye + H_2O

Procedure For Total bilirubin & Total Cholesterol

Pipette into tubes marked	Blank	Standard	Test
Serum/Plasma	-	-	10µL
Reagent 2	-	10µL	-
Reagent 1	1000µL	1000µL	1000µL

Mix well, Incubated at 37ºC for 10 minutes. Programme the analyser as per assay parameters. Blank the analyser with Reagent Blank. Measure absorbance at 505nm of Standard followed by the test. Calculated results as per given calculation formula.

Calculation

For Total Cholesterol

Cholesterol concentration (mg/dl) = Abs. of Test/Abs. of Standard × 200

Statistical analysis

The mean values _+SEM will be calculated for each parameter. The data will be analysed using one way analysis of variance (ANOVA).Post hock analysis will be done by Tukey's or Dunnet's test.

RESULT

Antioxidant ctivity (*In-vitro*)

The *in-vitro* antioxidant activity of Methanolic extracts of *Phyllanthus virgatus whole plant* was evaluated and the results obtained are as follows.

Influence of Methanolic extract of *Phyllanthus vargatus whole plant* on GSH levels in CCl_4 induced hepatotoxicity in rats

This was a marked reduction in the tissue GHS level i.e. to the extent o 47.56% up on CCl_4 treatment. Treatment with methanolic extract has brought back the decrease GSH levels in a dose dependant manner. However the effect of test extract was found to be more significance than the standard. The results were summarized in table.

Influence of Methanolic extract of *Phyllanthus vargatus whole plant* on lipid- peroxidation levels in CCl_4 induced hepatotoxicity in rats

There is a dose dependent inhibition of *in-vitro* lipid peroxidation by both doses of Methanolic extract. 50 mg/kg silymarin has 66.44% inhibition where has 400 mg/kg of Methanolic extract of the whole plant has 67.28% inhibition, which is superior to standard silymarin. The results are summarized in table.

Influence of Methanolic extract of *Phyllanthus vargatus whole plant* on GSH levels in paracetamol induced hepatotoxicity in rats

There is a marked depletion of GSH levels in paracetamol treated group. 50 mg/kg silymarin has increased it by 71.73%, Methanolic extract has shown a dose dependent increase in the levels of GSH, 400 mg/kg Methanolic extract has increased the GSH levels by 58.19% which is almost equal to standard silymarin. The results are summarized in Table

Influece of Methanolic extract of *Phyllanthus vargatus whole plant* on lipid peroxidation levels in paracetamol induced hepatotoxicity in rats

There is a dose dependent inhibition of *in-vivo* lipid peroxidation by both doses of Methanolic extract. 50 mg/kg of silymarin has 62.12% inhibition where 400 mg/kg of Methanolic extract of the whole plant has 58.75% inhibition, which isalmost equal to standard silymarin. The results are summarized in Table.

HEPATOPROTECTIVE ACTIVITY

Effect of Methanolic extract of *Phyllanthus vargatus whole plant* on biochemicalmarkers in CCl_4 induced hepatotoxicity

These increase in SGPT levels observed in CCl_4 treated group (60.38IU/L).The extract has shown a dose dependent effect. SGPT levels were restored to (58.33 IU/L by 400 mg/kg of Methanolic extract of the whole plant, which is near to effect of 50 mg/kg silymarin i.e.(57.68 IU/L).

SGOT levels has been increased significantly in CCl_4 treated group i.e. 572.93 IU/L, (200mg/kg 400 mg/kg) of Methanolic extract of the whole plant reduced the elevated levels ofSGOT to152.76 IU/L, which is near to silymarin effect of 206.78 IU/L.

In case of total bilirubin, a dose dependent effect of the extract is observed. 400 mg/kg of Methanolic extract has reduced the elevated levels of total by CCl_4 respectively. The results of (200mg/kg 400 mg/kg) Methanolic extract were found to be comparable with the results of 50 mg/kg silymarin on the same marker enzymes.

There is no significant rise of total cholesterol took place in CCl_4treated group. Dose dependent effect was observed with the Methanolic extract and result of (200mg/kg, 400mg/kg) of Methanolic extract is comparable with 50 mg/kg silymarin.

Effects of Methanolic extract of *Phyllanthus vargatus whole plant* on biochemical markers in Paracetamol induced hepatotoxicity

There is an increase in SGPT levels observed in paracetamol treated group (98.4 U/L). The extract has shown a dose dependent effect. SGPT levels were restored to 86.53 U/L by 200 mg/kg of Methanolic extract of the whole plant , which isnear to effect of 50mg/kg silymarin i.e. 75.75 U/L.

SGOT levels has been increased significantly in paracetamol treated group i.e. 170.58 U/L (200mg/kg,400mg/kg)of Methanolic extract of the whole plant reduced the elevated levels of SGOT to 176.86 U/L, which is near to silymarin effect of 166.11 U/L.In case of total Bilirubin a dose dependent effect of the extract is observed. ,(200mg/kg 400 mg/kg) Methanolic extract has reduced the elevated levels of total levels by paracetamol from respectively. The results of 200,400 mg/kg of Methanolic extract were found to be comparable with the results of 50 mg/kg silymarin on the same marker enzyme.

There is no significant rise of total cholesterol took place in paracetamol treated group.

Dose dependent effect was observed with the Methanolic extract and results of 200mg/kg ,400 mg/kg Methanolic extract is comparable with 50 mg/kg silymarin.

Table 1. **Effect of 70% Methanolic extract of *Phyllanthus vargatus whole plant* on tissue GSH levels in CCl_4 induced hepatotoxicity**

Treatment	Absorbance
Normal Control (1ml vehicle)	0.932 ± 0.03
CCl_4 (1ml/kg i.p)	0.536 ± 0.008
CCl_4 + Silymarin (1ml/kg i.p. + 50 mg/kg p.o)	0.899 + 0.04***
CCl_4 + Methanolic extract (1ml/kg i.p. + 200 mg/kg p.o)	0.691 + 0.03**
CCl_4 + Methanolic extract (1ml/kg i.p. + 400 mg/kg p.o)	0.890 ± 0.03***

Table 2. Effect of M**ethanolic extract of *Phyllanthus vargatus whole plant* on tissue lipid peroxidation levels in CCl_4 induced hepatotoxicity**

Treatment	Absorbance
Normal Control (1ml vehicle)	0.332 ± 0.14
CCl_4 (1ml/kg i.p)	0.693 ± 0.11
CCl_4 + Silymarin (1ml/kg i.p. + 50 mg/kg p.o)	0.299 + 0.008***
CCl_4 + methanolic extract (1ml/kg i.p. + 200 mg/kg p.o)	0.400 + 0.007*
CCl_4 + methanolic extract (1ml/kg i.p. + 400 mg/kg p.o)	0.294 + 0.006***

Values are the mean ± S.E.M. of six rats/treatment. Significance ***P< 0.001, **P< 0.01, *< 0.05, compared to CCl_4 treatment.

Table 3. Effect of M**ethanolic extract of *Phyllanthus vargatus whole plant* on tissue GSH levels in Paracetamol induced hepatotoxicity.**

Treatment	Absorbance Mean ± SEM
Normal Control (1ml vehicle)	0.949 ± 0.03
Paracetamol Treated (1gr/kg p.o)	0.521 ± 0.008
Paracetamol + Silymarin	0.823 + 0.006***
Paracetamol + Methanolic extract	0.652 + 0.006**
Paracetamol + Methanolic extract	0.766 + 0.005***

Values are the mean ± S.E.M. of six rats/treatment. Significance ***P< 0.001, **P< 0.01, *< 0.05, compared to Paracetamol treatment.

Table 4. Effect of M**ethanolic extract of *Phyllanthus vargatus whole plant* on tissue lipid peroxidation levels in Paracetamol induced hepatotoxicity;**

Treatment	Absorbance Mean ± SEM
Normal Control (1ml vehicle)	0.340 ± 0.029
Paracetamol Treated (1ml/kg p.o)	0.694 ± 0.026
Paracetamol + Silymarin	0.325 + 0.010***
Paracetamol + Methanolic extract	0.514 + 0.012**
Paracetamol + Methanolic extract (1gr/kg p.o. + 200 mg/kg p.o)	0.345 ± 0.024***

Values are the mean ± S.E.M. of six rats/treatment. Significance ***P< 0.001, **P< 0.01, *< 0.05, compared to Paracetamol treatment.

Table 5. Effect of Methanolic extract of *Phyllanthus vargatus whole plant* on hepatic enzymes in CCl_4 induced hepatotoxicity

Treatment		Treatment		Treatment	
	ALT or SGPT u/L		ALT or SGPT u/L		ALT or SGPT u/L
Normal control	72.05±0.79	Normal control	72.05±0.79	Normal control	72.05±0.79
Disease control	384.93±0.64***	Disease control	384.93±0.64***	Disease control	384.93±0.64***
Silymarine (50mg/kg)	57.68±0.19***	Silymarine (50mg/kg)	57.68±0.19***	Silymarine (50mg/kg)	57.68±0.19***
Low dose 200mg/kg	60.38±0.17***	Low dose 200mg/kg	60.38±0.17***	Low dose 200mg/kg	60.38±0.17***

*P<0.05, **P<0.01 and *** P<0.001 Significance compared to CCl_4 treatment.

Table 6. Effect of 70% Methanolic extract of *Phyllanthus vargatus whole plant* on hepatic enzymes in paracetamol induced hepatotoxicity

Treated groups	SGPT or ALT U/L	SGOT or AST	Total Bilirubin mg/dl	ALPu/L	Total Cholesterol mg/DL
Negative control	72.08±0.48	184.4±1.47	0.26±0.007	392.68±4.36	124.54±3.19
Positive control1gr/kg	384.81±0.34***	644.97±1.37***	1.53±0.03***	750.4±1.09***	168.38±8.89***
Silymarine100mg/kg	75.75±0.21***	166.11±0.91***	0.24±0.003***	325.93±3.47***	117.59±2.95***
Low dose 200mg/kg	98.4±0.08***	170.58±0.42***	0.49±0.004***	622.78±1.10***	155.21±1.89***
High dose 400mg/kg	86.53±0.10***	176.86±1.40***	0.57±1.01***	328.45±6.04***	128.19±2.82***

Values are the mean ± S.E.M. of six rats/treatment. *P> 0.05, ** P> 0.01 and *** P> 0.001 Significance compared to paracetamol treatment

DISCUSSION

Management of liver diseases is still a challenge to modern medicine. The methanolic extract was subjected to *in-vivo* antioxidant activity. Effect on GSH and lipid peroxidation content upon CCl_4 and paracetamol induced GSH and lipid peroxidation depletion. Per-treatment with methanolic extract has increased the depleted GSH and decreased lipid peroxidation levels during both CCl_4 and paracetamol challenges in a dose dependent manner. The lipids peroxidation is directly proportional to the tissue damage and extent of GSH depletion is also the indicator of severity of tissue damage. Therefore this extract was further subjected to screen for organ protective properties against various experimentally induced organ toxicities, e.g. hepatotoxicity (CCl_4 and paracetamol induced hepatotoxicity). The extract was subjected to screen for hepatoprotective activity against CCl_4 induced hepatotoxicity in rats. Administration of CCl_4 has caused the hepatotoxicity as indicated by the enhanced levels of biochemical markers of hepatotoxicity, e.g. SGPT, SGOT, ALP, Total bilirubin, and Total Cholesterol levels. Histopathology reports reveal that administration of CCl_4 has shown various degradation of fatty cysts, infiltration of lymphocytes, proliferation of kuffer cells and congestion of liver sinusoids. This further confirms that CCl_4 administration cause hepatotoxicity. Upon pre-treatment with methanolic extract has decreased the elevated levels of biochemical markers like SGPT, SGOT, ALP, Total bilirubin, Total cholesterol levels in a dose dependent manner. Similarly, histopathological observations show that hepatic globular architecture was normalized; fewer lymphatic infiltrations were seen kuffer cells proliferation appears normal. These observations suggest that the methanolic extract of whole plant of *phyllanthus virgatus* possess hepatoprotective activity against CCl_4 induced hepatotoxicity. It has normalized the CCl_4 induced biochemical and tissue aberrations, therefore it may be suggested that hepatoprotective activity against CCl_4 challenge is probably due to its free radical scavenging activity and prevention of lipid peroxidation. The restoration of tissue GSH and lipid peroxidation levels by the treatment with test extract is indicating that the on built protective mechanism is being restored. This hepatoprotective activity may be attributed to the anti-oxidant activity of the plant. However our studies do not confirm whether test extract blocks cytocrome $P4502E_1$ enzyme and thereby inhibit the formation of tricholomethoxy radical. In paracetamol induced hepatotoxicity also similar results were obtained i.e. treatment with paracetamol elevated the levels of marker enzymes like SGPT, SGOT, ALP, Total bilirubin total Cholesterol, increases tissue GSH and decreased

lipid peroxidation levels. Treatment with methanolic extract of the levels of *phyllanthus virgatus whole plant* have brought back the altered levels of the biochemical marker enzymes like SGPT, SGOT, ALP, Total bilirubin, Total Cholesterol, tissue GSH and lipid peroxidation levels to near normal levels. Even Histopathological studies have shown similar type of improvement in the anatomy of liver. It appears that the anti-oxidant property i.e. increased hydroxyl scavenging and super anion scavenging activities may be involved in the protective action of 70%methanolic extrat of levels of *phyllanthus virgatus*. These results are indicating that hepatoprotective activity of methanolic extract of whole plant *phyllanthus virgatus* against paracetamol induced hepatotoxicity may be due to scavenging free radicals. There reports that flavonoids and catechol derivatives like 1-nor-adrenaline, dopamine and dopa are present in the whole plant of *phyllanthus virgatus* are known to have very good antioxidant property. It reports that whole plant contain glutathione (GSH), which is a known anti-oxidant and tissue protective component. In addition, the tissue GSH levels were raised due to treatment with test extract (this rise may be due to GSH content of the leaves or due to prevention of its depletion by exogenous challenges). Therefore hepatoprotective activity of whole plant of *phyllanthus vargatus* may be attributed to these principles including GSH. However further studies are need to quantitatively assess the role of these principles in hepatoprotection.

CONCLUSION

The whole plant of *phyllanthus virgatus* contain saponins, tannins, flavonoids, carbohydrates, cardiac glycosides and proteins. The study was taken up to evaluate methanolic extracts of whole plant of *phyllanthus virgatus* for antioxidant and hepatoprotective activities. The acute toxicity study conducted for methanolic, extracts indicates that there are safe up to 2000mg/kg bodyweight. methanolic extract of the whole plant of *phyllanthus virgatus* has demonstrated dose dependent increase in the depletion tissues GSH and decrease lipid peroxidation levels by both CCl_4 and paracetamol .Treatment with 70%methanolic extract has brought back the elevated levels of SGPT, SGOT, Total cholesterol, Total and Direct Bilirubin, Cholesterol in both $CCl_4$and paracetamol induced hepatotoxicity in rats.

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