



Scholars Research Library

Der Pharma Chemica, 2013, 5(1):306-312
(<http://derpharmachemica.com/archive.html>)



ISSN 0975-413X
CODEN (USA): PCHHAX

Antioxidant and curative effect of *Monochoria vaginalis* methanolic extract against carbon tetrachloride induced acute liver injury in rats

B. Latha and M. S. Latha*

Biochemistry and Pharmacognosy Research Laboratory, School of Biosciences, Mahatma Gandhi University, P. D. Hills., Kottayam, Kerala, India

ABSTRACT

Aim of the present study was to investigate the antioxidant and curative effect of the methanolic extract of *Monochoria vaginalis* (MEMV) against Carbon tetrachloride (CCl_4) induced acute liver injury in rats in curative models. Toxic control, MEMV and standard drug Silymarin treated rats were received a single dose of CCl_4 (150 μ l/100 g, 1:1 in ground nut oil). In post-treatment groups, rats were treated with MEMV at doses of 100mg and 200mg/kg and Silymarin at a dose of (50mg/kg), 2, 24 and 48hrs after CCl_4 administration. Rats treated with the extract after the establishment of CCl_4 induced liver damage showed significant ($p \leq 0.05$) protection of liver as evidenced from normal AST, ALT, ALP and lipid peroxide levels. Hepatic glutathione levels were significantly ($P \leq 0.05$) increased by the treatment. Histopathological changes induced by CCl_4 were also significantly ($p \leq 0.05$) reduced by the extract treatment in curative groups. The antioxidant status of the animals were also assessed by measuring the activity of GSH, Catalase, SOD, GST and GPx. The extent of lipid peroxidation was also measured. Phytochemical screening of this plant revealed the presence of flavonoids and alkaloids which could be responsible for the possible hepatoprotective action. This study demonstrates the antioxidant and curative effect of MEMV and thus scientifically proves the use of entire plant in traditional medicine for hepatic disorders.

Key words: *Monochoria vaginalis*, carbon tetrachloride, hepatotoxicity, lipid peroxidation

INTRODUCTION

Herbal drugs are being proved as effective as synthetic drugs with lesser side-effects. *Monochoria vaginalis* is a plant belonging to the pontederiaceae family known to have several medicinal properties[1]. It is a weed found in rice (*Oryza sativa*) fields and is used as a vegetable. Eventhough the plant root stock and leaves have cooling, bittersweet, aromatic, alterant and diuretic properties, some of the phytochemical and pharmacological properties in this plant still remain unexplored. The leaf juice of *Monochoria vaginalis* is used to treat cough and that of roots is used to treat stomach and liver problems, asthma and tooth ache[2,3].

Many folk remedies from plant origin are evaluated for its possible antioxidant and hepatoprotective effects against different chemical-induced liver damage in experimental animals. CCl_4 -induced hepatotoxicity model is frequently used for the investigation of hepatoprotective effects of drugs and plant extracts[4,5].

Most of these compounds prevent peroxidative damage to liver microsomes and hepatocytes[6]. The main objective of the study was to evaluate the antioxidant and curative effect of Methanolic extract of *M. vaginalis* (MEMV)

against liver disorders. In the present investigation the antihepatotoxic activity of MEMV was conducted in acute liver injury model against CCl₄ in curative treatments.

MATERIALS AND METHODS

Chemicals

Carbon tetrachloride was purchased from Merck, Mumbai. Silymarin was purchased from sigma chemical Co., St. Louis, MO, USA. 5,5'-dithiobis-(2-nitrobenzoic acid), 1-chloro-2,4-dinitrobenzene was purchased from Sisco Research laboratories, Mumbai, India. Assay kits for serum marker enzymes AST, ALT, ALP were purchased from span diagnostics Ltd., Surat, India. All other chemicals were of analytical grade.

Collection of plant material and preparation of extracts

Monochoria vaginalis was collected from Kumarakom, kottayam, kerala. The root stock was washed thoroughly and dried at room temperature in shade and authenticated. A voucher specimen (SBSBRL.15) is maintained in the institute. A 100gm of dried powder of the plant was soxhlet extracted with 500ml of methanol for 48 hr. The step was repeated with a new set of dried powder and solvent until the required quantity was achieved. The extract was concentrated in a rotary evaporator and yield recorded was 5.06%. It was suspended in 5% Tween80 and was stored in refrigerator till further use.

Preliminary phytochemical studies

Phytochemical screening of the extract was performed by using the following reagents and chemicals. Alkaloids by chemical tests (alkaloid fraction gave positive test for Meyers reagent, Dragendorff reagent and Wagner reagent; flavonoids with the use of concentrated HCl and magnesium turnings, tannins with 5% ferric chloride, saponins with ability to produce suds, gum with molisch's reagent and concentrated sulphuric acid, steroids and terpenoids with chloroform and concentrated HCl, phytosterols with Liebermann's Burchard test, reducing sugars with the use of alpha naphthol and sulphuric acid, protein and free amino acid by millons, biuret and ninhydrin test. Various phytochemical investigation studies shows the presence of alkaloids, flavonoids and saponins in MEMV.

In vivo antioxidant studies

Animals and diets

Male wistar rats (150-200g) were used for the study. The animals were housed in well ventilated cages and given standard rat chow (Sai Feeds, Bangalore, India), and water ad libitum. The animals were maintained at a controlled condition of temperature of 26-28°C with a 12 hrs light: 12 h dark cycle. Animal studies were followed according to Institute Animal Ethics Committee (IEAC) regulations approved by Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) (Reg. No.B 2442009/5) and conducted humanely.

Acute oral toxicity

Acute oral toxicity of MEMV was determined using wistar albino rats. The overnight fasted rats were weighed and selected. Methanolic extract was dosed in a stepwise procedure, with the initial dose being selected as the dose expected to produce some signs of toxicity and were observed for a period of two weeks. LD₅₀ values of the methanolic extract was (1000mg/kg,po), hence 100mg/kg was considered as effective for the hepatoprotective activity. Selected doses of MEMV were 100mg/kg and 200mg/kg.

Experimental design

In the post-treatment, animals were divided into five groups with six rats in each group. Group I was normal control. Group II to V animals were administered orally with a single dose of CCl₄ (150µl/100g of 1:1 mixture in ground nut oil) on day 1. Groups III to V received 100 and 200mg/kg MEMV and Silymarin (50mg/kg), respectively 2, 24 & 48h after CCl₄ administration.

Biological assay

Serum enzyme analysis

Blood was collected from the neck blood vessels under mild ether anaesthesia and kept for 30min at 4°C. Serum was separated by centrifugation at 2500rpm at 4°C for 15min. The liver was removed rapidly and cut into separate portions for hepatic glutathione, antioxidant enzymes, lipid peroxide estimation and histopathological studies. AST (E.C 2.6.1.1), ALT (E.C 2.6.1.2) and ALP (E.C 3.1.3.1) levels were assayed by using span diagnostic kits by

kinetic method using semi autoanalyzer (RMS, India). Antioxidants and lipid peroxidation levels were measured photometrically.

Tissue analysis

Liver was excised, washed thoroughly in ice- cold saline to remove the blood stain. Ten percent of liver homogenate was prepared in 0.1 M tris HCl buffer (pH-7.4). The homogenate was centrifuged at 3000 rpm for 20 min at 4°C and the supernatant was used for the estimation of Superoxide Dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx), glutathione-S-transferase (GST), reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS).

Reduced glutathione was determined according to Moron *et al* [7] method using Ellmann's reagent. The procedure is based on the reduction of Ellmann's reagent by SH groups to form 5, 5'-dithiobis(2-nitrobenzoic acid), the intensity of yellow colour developed was measured spectrophotometrically at 412 nm using a spectrophotometer. Results were expressed as nmol

GSH/mg protein. SOD (E.C 1.15.1.1) was assayed by following the method of Kakkar *et al* [8]. Activity of SOD was expressed as units/mg protein. The CAT (E.C 1.11.1.6) activity was determined from the rate of decomposition of H₂O₂ according to the method of Cohen *et al* [9]. A 0.3 ml of supernatant was added to 3ml of 0.067 M phosphate buffer (pH 7.0) and 0.75ml of H₂O₂. The rate of decomposition of H₂O₂ was measured spectrophotometrically at 240nm. Activity of catalase was expressed as U/mg of protein. GPx (E.C 1.11.1.9) activity was determined by measuring the decrease in GSH content after incubating the sample in the presence of H₂O₂ and NaN₃. Activity of GPx was determined by the method of Rotruck *et al* [10] and was expressed as μmoles of GSH oxidized/min/mg protein. GST (E.C 2.5.1.18) activity was determined from the rate of increase in conjugate formation between reduced glutathione and CDNB by Beutler [11].

Lipid peroxidation levels in liver homogenate was estimated by Nichan's and Samuelson method [12]. In brief, the amount of malondialdehyde (MDA) was measured in terms of thiobarbituric acid reactive substances (TBARS), which is undertaken as an index of lipid peroxidation using 1,1',3,3' tetramethoxypropane as standard [13]. Results were expressed as nmol MDA/mg protein for TBARS. Protein content in the tissue was determined using bovine serum albumin (BSA) as standard [14].

Histopathological studies

Small pieces of liver, fixed in 10% buffered formalin were processed for embedding in paraffin [15]. Sections of 5-6 μm were cut and stained with hematoxylin and eosin and examined for histopathological changes under the microscope (Motic AE 21, Germany). The micrographs were taken using Moticam 1000 camera at original magnification of 100X.

Liver sections were graded numerically to assess the degree of biological features in acute hepatic injury. Centrilobular necrosis, characterised by the damage of several liver cells around the central vein, fatty infiltration, prominent ballooning etc. and bridging hepatic necrosis, a form of confluent necrosis of liver cells linking central veins to portal tracts or portal tracts to one another were prominent in histological findings [16]. A combined score of centrilobular necrosis & bridging hepatic necrosis was given a maximum value of 6. Descriptive modifiers such as mild, moderate & severe was applied to activity and staging. The parameters were graded from score 0 to 6, with 0 indicating no abnormality, 1 to 2 indicating mild injury, 3 to 4 indicating moderate injury and 5 to 6 with severe liver injury.

Statistical analysis

Results were expressed as mean ± S.D and all statistical comparisons were made by means of one way ANOVA test followed by Tukey's post hoc analysis and P values less than or equal to 0.05 were considered significant

RESULTS

Serum enzyme analysis

In the post-treatment groups, rats administered with CCl₄ showed an elevated levels of serum AST, ALT, ALP and lipid peroxides. Treatment with the extract of *M.vaginalis* at doses of 100mg and 200mg/kg significantly (p≤0.05)

protected the rats from CCl₄ induced hepatotoxicity. Standard drug Silymarin also showed a remarkable protection towards CCl₄ administration. Treatment with 100mg and 200mg/kg MEMV and Silymarin (50mg/kg) exhibited a protection of 96.1, 98.8 and 97.1% on AST levels, and 96.5, 98.5 and 96.9% in ALT levels and 94.5, 96.9 and 95% ALP levels respectively (Table 1).

Table-1: Effects of MEMV and Silymarin post-treatment on changes of Biochemical parameters

TREATMENT GROUPS	AST (IU/L)	ALT (IU/L)	ALP (IU/L)	TBARS
NORMAL CONTROL	155.8 ± 3.5	64.6 ± 3.5	150.9 ± 3.6	2.01 ± 1.61
CCl ₄ CONTROL (150 µl/100g)	639 ± 20.1 [#]	583.1 ± 14.1 [#]	676.1 ± 19.5 [#]	15.95 ± 2.12 [#]
SILYMARIN 50mg/kg+CCl ₄)	172.3 ± 5.1 [*]	84.3 ± 4.2 [*]	295.3 ± 10.3 [*]	2.82 ± 2.3 [*]
M. VAGINALIS (100mg/kg+CCl ₄)	178.2 ± 5.5 [*]	90.1 ± 6.2 [*]	298.2 ± 10.6 [*]	2.91 ± 2.21 [*]
M. VAGINALS (200mg/kg+CCl ₄)	164.1 ± 3.4 [*]	77.1 ± 5.3 [*]	261.5 ± 9.9 [*]	2.31 ± 1.71 [*]

Values are mean ± S.D., n = 6 ; [#]P ≤ 0.05 V_s normal control; ^{*}P ≤ 0.05 V_s CCl₄ control
TBARS: n moles of MDA/gm tissue

Tissue analysis

Estimation of lipid peroxides

A significant increase (p ≤ 0.05) in tissue lipid peroxide level (TBARS) was observed in CCl₄ alone treated rats. However CCl₄ induced elevation of TBARS concentration were lowered significantly (p ≤ 0.05) by the post-treatment of the rats with the extract. In MEMV treated rats, TBARS levels were remarkably reduced which was comparable to Silymarin (Table 1).

Estimation of reduced (GSH), GST, GPx, SOD and Catalase

In the post-treatment groups, rats administered with CCl₄ alone were found significantly (p ≤ 0.05) lowered the hepatic GSH, GST, GPx, SOD and Catalase levels. Various doses of MEMV and Silymarin treated rats completely prevented the lowering of above mentioned parameters (Table 2).

Table-2 : Effect of Methanolic extracts of Monochoria Vaginalis and silymarin on antioxidant levels

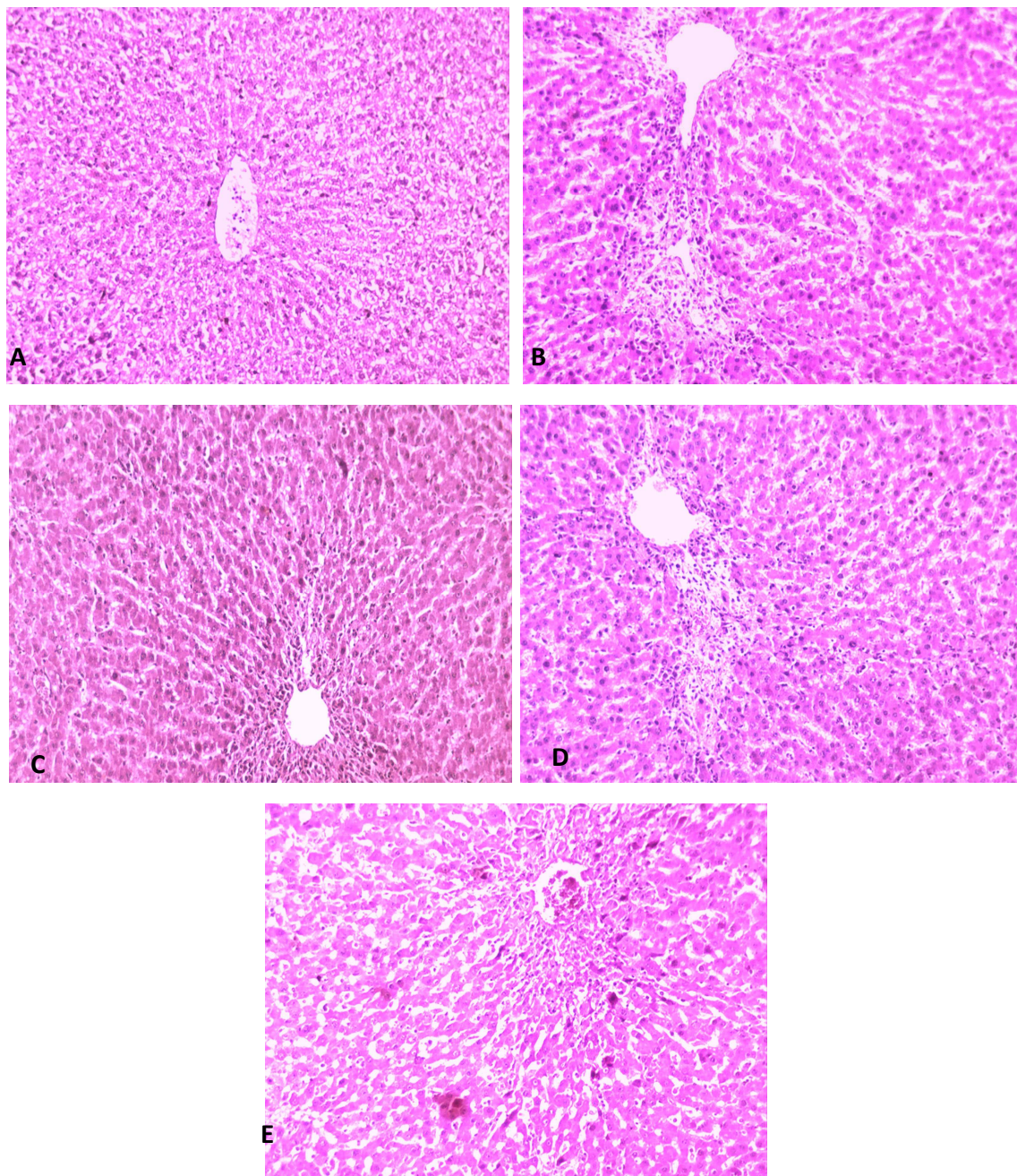
TREATMENT GROUPS	GSH	SOD	CAT	GPx	GST
NORMAL CONTROL	2.33 ± 0.29	166.2 ± 10.1	33.26 ± 3.9	39.23 ± 2.01	0.49 ± 0.03
CCl ₄ CONTROL (150 µl/100g)	0.70 ± 0.05 [#]	92.51 ± 8.5 [#]	20.1 ± 2.85 [#]	17.42 ± 3.05 [#]	0.17 ± 0.02 [#]
SILYMARIN (50mg/kg+CCl ₄)	2.22 ± 0.28 [*]	135.1 ± 8.2 [*]	27.72 ± 3.2 [*]	31.19 ± 3.31 [*]	0.35 ± 0.02 [*]
M. VAGINALIS (100mg/kg+CCl ₄)	1.98 ± 0.19 [*]	132.9 ± 7.85 [*]	26.91 ± 3.3 [*]	29.21 ± 3.02 [*]	0.32 ± 0.01 [*]
M. VAGINALIS (200mg/kg+CCl ₄)	2.34 ± 0.25 [*]	161.3 ± 8.01 [*]	29.38 ± 2.9 [*]	35.21 ± 2.51 [*]	0.45 ± 0.02 [*]

Values are mean ± S.D., n = 6 ; [#]P ≤ 0.05 V_s normal control; ^{*}P ≤ 0.05 V_s CCl₄ control;

GSH: nmole/mg protein; SOD: Units/min/mg protein; CAT: µ moles of H₂O₂ consumed/min/mg protein; GPx: Moles of GSH oxidized/min/mg protein; GST: µ moles of CDNB-GSH conjugate formed/min/mg protein.

Histopathological analysis

In rats treated with carbontetra chloride, the normal architecture of liver (figure 1B) was completely lost with the appearance of centrilobular necrosis and bridging hepatic necrosis scoring 5.4 ± 0.4 (mean ± S.D ; n=3) (fig 1). The post-treatment animals administered with plant extract at 100 and 200 mg/kg and Silymarin (50mg/kg) showed a significant (p ≤ 0.05) protection from CCl₄ induced liver damage as evident from hepatic architectural pattern with mild to moderate hepatitis with scores 3.45 ± 0.5; 2.85 ± 0.65; 3.4 ± 0.6 (mean ± S.D ; n=3; p ≤ 0.05) respectively (figure 1 C -2E).

Fig 1 : Histopathological changes during carbontetra chloride (CCl₄) intoxicationFig1:(A)Normal control; (B)CCl₄ control; (C) CCl₄+Silymarin(50mg/kg);(D)CCl₄+MEMV 100mg/kg);(E)CCl₄+MEMV(200mg/kg).

DISCUSSION

CCl₄ has been widely used in animal models to investigate chemical toxin –induced liver damage. The most remarkable pathological characteristics of CCl₄ -induced hepatotoxicity are fatty liver, cirrhosis and necrosis, which have been thought to result from the formation of reactive intermediates such as trichloromethyl free radicals (CCl₃^{*}) metabolized by the mixed function cytochrome p₄₅₀ in the endoplasmic reticulum[17]. Usually the extent of hepatic damage is assessed by the increased level of cytoplasmic enzymes (AST, ALT and ALP), thus leads to

leakage of large quantities of enzymes into circulation. This was associated by massive centrilobular necrosis, ballooning degeneration and cellular infiltration of the liver[18].

Aminotransferases are present in high concentration in liver, an important class of enzymes linking carbohydrate and amino acid metabolism. Alanine aminotransferase and aspartate aminotransferase are well known diagnostic indicators of liver disease. Alkaline phosphatase, a membrane bound enzyme and its elevations in plasma indicate membrane disruption in the organ. Alkaline phosphatases, although not a liver specific enzyme, liver is the major source of this enzyme. Measurement of these enzymes levels has proved to be powerful tools in the assessment of hepatotoxicity[19]. The results obtained clearly depict the extent of damage created by CCl₄ to the hepatic tissue.

Administration of plant extract significantly prevented CCl₄ induced elevation of AST, ALT and ALP indicating the hepatoprotective activity of this herb. In a dose response study, it was found that the extract at a dose of 200mg/kg could completely protect the liver from toxic injury. *M.vaginalis* extract at a dose of 100mg/kg was comparable to standard control drug, Silymarin also showed better protection towards CCl₄ induced liver damage. The methanolic extract significantly reduced the elevation of lipid peroxide levels which was comparable to silymarin. This extract also prevented the decrease of hepatic GSH, GST, GPx, SOD and catalase in CCl₄ induced rats and retained the levels to standard control rats, indicating the antioxidant activity of the extract. Antioxidant enzymes represent one protection against oxidative damage[20]. SOD converted O₂ into H₂O₂ and Catalase metabolize H₂O₂ to non-toxic products. The GSH antioxidant system plays a fundamental role in cellular defense against reactive free radicals and other oxidant species. Hepatic GSH, GST, GPx, SOD, Catalase and MDA formation were completely restored to normal values by the treatment with the extract and Silymarin. Antioxidant property is claimed to be one of the mechanism of hepatoprotective drugs.

The histological changes induced by CCl₄ treatment as evidenced by centrilobular necrosis and bridging hepatic necrosis and its protection to normalcy by the treatment with the plant extract was indicative of the hepatoprotection of the extract. Histopathological evaluation showed negligible damage to a few hepatocytes present in the close vicinity of central vein in extract treated rats and the improvement of histological scores proved the efficacy of the extract as an antihepatotoxic agent.

The phytochemical studies reveal the presence of flavonoids (3-O-beta-glucopyranoside). Fraction of n-butanol from *Monochoria vaginalis* exhibited anti-oxidant activity[21]. Seven phytoconstituents were identified from ethanol extract of *Monochoria vaginalis* by using Gas Chromatograph-Mass Spectrograph(GCMS). The result of serum biochemical parameters, level of hepatic lipid peroxides, hepatic glutathione, antioxidant enzymes and histopathological studies in the post-treatment groups support the hepatoprotective and antioxidant activity of MEMV.

CONCLUSION

The present study scientifically validates the hepatoprotective efficacy of *M.vaginalis*. Histological changes supported the findings. So *M.vaginalis* could be used for the development of phytomedicines against hepatic disorders and oxidative damage.

Acknowledgments

Financial assistance from Mahatma Gandhi University as Junior Research Fellowship to Latha.B is thankfully acknowledged.

REFERENCES

- [1]. Sie Monsma J. *Monochoria Vaginalis*, Eco Crop Code 1485(1993): 210-212
- [2]. Yoganarasimmen SN, *Medicinal Plants of India*. 2, Cyber Media, Bangalore, 2000. p.282
- [3]. Madhava Chetty K, Siraj K and Tulasi Rao K. *Flowering plants of Chittoor District*. Student Press, Andhra Pradesh, India, 2008. p.360
- [4]. Rubinstein D, *American journal of physiology.*, 1962, 203,1033-1037.
- [5]. Suja SR, Latha PG, Pushpangadan P, Rajasekharan S. *Journal of Tropical Medicinal Plants.*, 2002, 3,191-195.
- [6]. Wang IK, Lin-Shieu SM, Lin JK. *European Journal of Cancer.*, 1999, 35, 1517-1525.

-
- [7]. MS Moron, JW Depierre, B Mannervick. *Biochem Biophys Acta.*, **1979**, 582, 67-78.
- [8]. Kakkar P, Das B and Viswanathan PN. *Ind.J.Biochem.Biophys .*, **1984**, 21,130-132.
- [9]. Cohen G,Dembiec D and Marians. *J.Anal.Biochem.*, **1970**, 34, 30-38.
- [10]. Rotruck JJ, Pope AL, Gantha HE and Swanson AB. *Science .*, **1973**, 179, 588-590 .
- [11]. Beutler E. Red cell metabolism. Churchill Livingston pub, Newyork ;**1986**. 51-53.
- [12]. Nichans WG and Samuelson B. *Eur.J .Biochem*,**1968**,6,126- 130.
- [13]. Hwang YP, Choi JH, Jeong HG. **2009**, *Food Chemtoxicol*, 47 (1): 75-81.
- [14]. Lowry OH, Rosebrough, AL Farr and RJ Randall. *J. Biol.Chem*, **1951**, 193, 267-75.
- [15]. Galigher AE, Kozloff EN. *Essentials of Practical Microtechnique*, Philadelphia, (**1971**), Lea & Febiger, p. 531.
- [16]. Lefkowitz JH. Pathologic diagnosis of liver disease.IN:Zakim D,Boyer TO.(Eds.) *Hepatology – A textbook of liver disease*. W.B.Saunders Company, Philadelphia (**1996**), p. 844-871.
- [17]. Recknagel RO, Glende Jr. Dolak JA, Waller RL. *Pharmacol.Ther* , **1989**, 43, 139-15.
- [18]. Gouri Shankar NL,Manavalan R, Venkappaya D, David Raj C. *Food Chem Toxicol*, **2008**,46,3182-3185.
- [19]. Victor W, Rodwell. General properties of enzymes. In: *Harper's review of Biochemistry*. Lange Medical Publications (**1981**) p.51-63.
- [20]. Halliwell B,Gutteridge JMC.Role of free radicals and catalytic metal ions in human disease, An Overview. *Methods Enzymol* ,**1990**, 186, 59-85.
- [21]. Zhou YJ, Xu, Xu, Qiao FY, Zhang J.P, Yu CQ. Isolation and identification of an antioxidant from *Monochoria vaginalis*.Ying Yong Shery Tai Xue Bao, **2007**, 509-513.