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Research Article

Hepatoprotective and antioxidant effect of *Polygala chinensis* L. whole plant against CCl_4 induced hepatotoxicity in rats

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Abstract

Background and objectives: Carbon tetrachloride (CCl_4) is one of the most commonly used hepatotoxins in the experimental study of liver diseases. Liver diseases are still a world wide health problem. Unfortunately, conventional and synthetic drugs used in the treatment of liver diseases are inadequate and sometimes can have serious side effects. There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity. The ethanol extract of whole plant of *Polygala chinensis* protect the liver from CCl_4 hepatotoxins.

Methods: The CCl_4 intoxicated adult male albino rats were treated with the ethanol extract of whole plant of *Polygala chinensis* in doses of 100 and 200 mg/kg orally for 14 days. The rats were sacrificed at the end of the 14 days. Biochemical parameters and antioxidant activities were carried out in the serum of control, CCl_4 intoxicated and drug treated rats.

Result: CCl_4 intoxicated rats showed significant elevation in serum enzymes, bilurubin and lipid peroxidation of the liver tissues and reduction in serum total protein, superoxide dismutase, catalase, reduced glutathione and glutathione peroxidase activity. Treatment with ethanol extract of *Polygala chinensis* whole plant altered the above parameters to the levels of near normal. All the above results were comparable with the standard drug silymarin (100 mg/kg) treated group.

Conclusion: The present study ascertains that the ethanol extract of *Polygala chinensis* whole plant possesses significant hepatoprotective activity.

Keywords: *Polygala chinensis,* Hepatoprotective activity, Antioxidant, CCl_4, Bilirubin, Silymarin

1. Introduction

Liver is a versatile organ of the body that regulates internal chemical environment. Liver injury induced by various hepatotoxins has been recognized as a major toxicological problem for years. Because of its unique metabolic functions and relationship to the gastrointestinal tract, liver is an important target of toxicity to xenobiotics, oxidative stress, ethanol and toxic chemicals (antibiotics, chemotherapeutics, aflatoxins, carbon tetrachloride, chlorinated hydrocarbons, etc.). There are numerous plants and polyherbal formulations claimed to have hepatoprotective activity. Nearly 150 phytoconstituents from 101 plants have been claimed to possess liver protecting activity [1].

Polygala was traditionally used by Native Americans to treat snake bites[2] and as an expectorant to treat cough and

bronchitis. In traditional Chinese medicine, *Polygala* is used for a variety of purposes including the promotion to sleep and calming the spirit. *Polygala* considered as a powerful tonic herb [3] that can help to develop the mind and aid in creative thinking. Biological activities such as antidiabetic, anti-inflammatory and antioxidant activities were reported[4,5,6]. However, so far there is no systematic study on hepatoprotective activity has been reported in the literature. Hence, the aim of the present study was to investigate the hepatoprotective activity of *P. chinensis* whole plant extract on CCl_4 induced liver toxicity in rats.

2. Material and Methods

2.1. Plant material

The well grown whole plant of *Polygala chinensis* L. was collected from Vadavalli, Coimbatore, Tamil Nadu. The collected plants were identified by the Botanical Survey of India, Coimbatore. A voucher specimen (VOCB 1348)was retained in Ethnopharmacology unit, Research Department of Botany, V.O.Chidambaram College, Tuticorin for further reference.

2.2.Preparation of plant extracts for phytochemical screening and hepatoprotective studies

The whole plant was dried under shade and then powdered with a mechanical grinder to obtain a coarse powder, which was then subjected to extraction in a Soxhlet apparatus using ethanol. The extract was subjected to qualitative test for the identification of various phytochemical constituents as per standard procedures[7,8,9]. The ethanol extracts were concentrated in a rotary evaporator. The concentrated extract was weighed to calculate the yield of ethanol (10.20%). The concentrated ethanol extract were used for hepatoprotective studies.

2.3.Animals

Normal healthy male Wistar albino rats (180-240 g) were used for the present investigation. Animals were housed under standard environmental conditions at room temperature (25±2°C) and light and dark (12:12 h). Rats were fed with standard pellet diet (Goldmohur brand, MS Hindustan lever Ltd., Mumbai, India) and water *ad libitum.* Study was carried out as per IAFC approval no:82/PHARMA/SCRI, 2010.

2.4.Acute Toxicity Studies

Acute oral toxicity study was performed as per OECD-423 guidelines (acute toxic class method), albino rats (n=6) of either sex selected by random sampling were used for acute toxicity study[10]. The animals were kept fasting for overnight and provided only with water, after which the extracts were administered orally at 5mg/kg body weight by gastric intubations and observed for 14 days. If mortality was observed in two out of three animals, then the dose administered was assigned as toxic dose. If mortality was observed in one animal, then the same dose was repeated again to confirm the toxic dose. If mortality was not observed, the procedure was repeated for higher doses such as 50, 100 and 2000 mg/kg body weight.

2.5.Experimental Design

In the investigation, a total of 25 rats (CCl_4 hepatic toxicity induced rats and 5 normal rats) were taken and divided into five groups of 5 rats each.

Group I: Rats received normal saline was served as a normal control.

Group II: CCl_4 hepatic toxicity induced control: Rats received 2.5ml/kg body weight of CCl_4 for 14 days.

Group III: Liver injured rats received ethanol extract of whole plant of *P. chinensis* at the dose of 100mg/kg body weight for 14 days.

Group IV: Liver injured rats received ethanol extract of whole plant of *P. chinensis* at the dose of 200mg/kg body weight for 14 days.

Group V: Liver injured rats received standard drug silymarin at the dose of 100mg/kg body weight for 14 days.

2.6. Biochemical Analysis

The animals were sacrificed at the end of experimental period of 14 days by decapitation. Blood was collected, sera separated by centrifugation at 3000 g for 10 minutes. Serum protein[11] and serum albumins was determined quantitatively by colorimetric method using bromocresol green. The total protein minus the albumin gives the globulin. Serum glutamate

pyruvate transaminase (SGPT), serum glutamate oxaloacetate transaminase (SGOT), serum alkaline phosphatase (ALP), total, conjugated bilirubin, unconjugated bilirubin were determined as per the standard procedures [12,13]. Liver homogenates (10%W/V) were prepared in ice cold 10mM tris buffer (pH7.4). Quantitative estimation of MDA formation was done by determining the concentration of thiobarbituric acid reactive substances (TBARS) in 10% liver homogenates by the method of Pal *et al*[14]. Antioxidants such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione reductase (GRD) were also assayed in liver homogenates as per the standard procedures [15, 16].

2.7.Statistical Analysis

The data were expressed as the mean ± S.E.M. The difference among the means has been analyzed by one-way ANOVA. $p<0.05$and $p<0.01$ were considered as statistical significance using SPSS Software.

3. Results

The ethanol extract of whole plant of *Polygala chinensis* subjected for phytochemical study showed the presence of alkaloids, coumarin, glycosides, flavonoids, saponins, steroids, phenols, tannins and xanthoproteins. The ethanol extract did not show any sign and symptoms of toxicity and mortality upto 2000 mg/kg dose. The effect of ethanol extract of *P. chinensis* on serum total protein, albumin, globulin, A/G ratio, serum transaminases and alkaline phosphatases in CCl_4 intoxicated rats are summarized in Table 1. There was a significant ($p< 0.01$) increase in serum GOT, GPT and ALP levels in CCl_4 intoxicated group (Group II) compared to the normal control group (Group I). The total protein and albumin levels were significantly ($p< 0.01$) decreased to 6.18 g/dl and 3.28 g/dl in CCl_4 intoxicated rats from the levels of 7.98 g/dl and 4.88 g/dl respectively in normal group. Ethanol extract of *P. chinensis* whole plant at the dose of 100 and 200 mg/Kg orally significantly decreased the elevated serum marker enzymes and reversed the altered total protein and albumin to almost normal level.

Table 1. Effect of whole plant extracts of *Polygala chinensis* on the protein, albumin, globulin concentration and enzyme activity of serum GOT, GPT, and ALP in the normal, liver damaged and drug treated rats.

Groups	Parameters						
	T.Protein (mg/dl)	Albumin (g/dl)	Globulin (g/dl)	A/G Ratio	SGOT (U/L)	SGPT (U/L)	ALP (U/L)
I	7.98±0.81	4.88±0.34	3.1±0.11	1.5:1	19.56±1.36	26.16±0.93	143.29±5.32
II	6.18±0.34*	3.28±0.11*	2.9±0.23	1.1:1	40.11±1.21*	43.19±1.08*	196.11±6.84*
III	7.21±0.18*a	4.46±0.16	2.75±0.12*	1.6:1	24.91±1.14*a	28.31±1.53**a	146.22±6.16*a
IV	7.96±0.19**a	4.37±0.14*a	3.59±0.11*	1.2:1	20.27±1.08**a	30.44±1.09*a	151.93±5.18*a
V	7.48±0.11*	4.51±0.31*	2.97±0.16	1.5:1	21.33±1.19*a	27.06±1.33*a	146.55±6.94*a

Each Value is SEM± 5 individual observations *P <0.05; ** P<0.01 Compared normal control vs liver injured rats a P<0.05;aa P<0.01 Compared liver injured rats vs drug treated

The effect of ethanol extract of *P. chinensis* on total, conjugated and unconjugated bilirubin is shown in Table 2.

Table 2. Effect of whole plant extracts of *Polygala chinensis* on the serum Total, conjugated and unconjugated bilirubin levels in the normal control, liver injured and drug treated rats.

Groups	Parameters		
	Total Bilirubin (µmol/L)	Conjugated (µmol/L)	Unconjugated (µmol/L)
I	0.68±0.03	0.24±0.01	0.44±0.02
II	3.69±0.43**	1.49±0.03*	2.20±0.06**
III	1.16±0.05*	0.12±0.01	1.04±0.06*
IV	0.81±0.03**	0.22±0.03**	0.59±0.02**
V	0.88±0.01**a	0.20±0.01*a	0.68±0.3*aa

Each Value is SEM± 5 individual observations *P<0.05; ** P<0.01 Compared normal control vs liver injured rats a- P<0.05; aa P<0.01 Compared liver injured rats vs drug treated

A significant elevation of total, conjugated and unconjugated bilirubin in the serum of CCl_4 intoxicated group (Group II) when compared to normal control (Group I). The ethanol extract of *P. chinensis* at the dose 100 and 200 mg/kg reduced the levels of total, conjugated and unconjugated bilirubin (Group III and Group IV). The decreases in the concentration of total bilirubin, conjugated bilirubin and unconjugated bilirubin were found to be greater in standard silymarin (Group V) followed by Group IV and Group III (Table 2).

The effects of ethanol extract of *P. chinensis* on lipid peroxidation (LPO), Glutathione peroxidase (GPx), Glutathione reductase (GRD), Superoxide dismutase (SOD) and Catalase (CAT) activity is shown in Table 3.

Lipid peroxidation level was significantly (p<0.01) increased and glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase activity were significantly (p< 0.01) decreased in CCl_4 intoxicated rats when compared with those of the animals in normal control group. Rats treated with ethanol extract of *P. chinensis* at the doses of 100 and 200 mg/kg significantly decreased the elevated lipid peroxidation levels and restored the altered glutathione peroxidase, glutathione reductase, superoxide dismutase and catalase levels towards the normal levels in a dose dependent manner. The results are well comparable with silymarin (standard drug) treated group.

Table 3. Effect of whole plant extracts of *Polygala chinensis* on liver LPO, GPX, GRD, SOD and CAT in the normal control, liver injured and drug treated rats.

Groups	Parameters				
	LPO (n mole of MDA/mg protein)	GPX (u/mg protein)	GRD (u/mg)	SOD (u/mg)	CAT (u/mg)
I	0.82±0.03	13.54±1.23	8.56±0.59	10.45±0.19	9.56±0.82
II	3.16±0.54**	4.22±0.56**	3.05±0.28*	4.22±0.28**	2.87±0.70**
III	1.32±0.21a	10.56±0.22a	6.98±0.84 a	5.97±0.16*	10.24±0.47aa
IV	0.94±0.17aa	14.59±1.04aa	8.85±0.23aa	8.56±0.52a	12.31±0.41aa
V	0.89±0.11aa	12.56±0.97aa	8.67±0.11aa	11.71±0.12aa	13.56±0.55aa

Each Value is SEM± 5 individual observations * P <0.05; ** P<0.01 Compared normal control vs liver injured rats a P<0.05; aa$P$<0.01 Compared liver injured rats vs drug treated

4. Discussion

It is well established that CCl_4 induces hepatotoxicity by metabolic activation; therefore it selectively causes toxicity in liver cells maintaining semi-normal metabolic function. CCl_4 is bio-transformed by the cytochrome P_{450} system in the endoplasmic reticulum to produce trichloromethyl free radical (CCl_3). Trichloromethyl free radical then combined with cellular lipids and proteins in the presence of oxygen to form a trichloromethylperoxyl radical, which may attack lipids on the membrane of endoplasmic reticulum faster than trichloromethyl free radical. Thus, trichloromethyl free radicals lead to elicit lipid peroxidation, the destruction of Ca^{2+} homeostasis and finally, results in cell death[17,18]. These results in changes of structures of the endoplasmic reticulum and other membrane, loss of enzyme, metabolic enzyme activation, reduction of protein synthesis and loss of glucose-6-phosphate activation, leading to liver damage[19, 20]. Hepatotoxic compounds like CCl_4 are known to cause marked elevation in serum enzyme activities. In the present study, treatment with *P. chinensis* whole plant extract attenuated the increase in the activities of SGOT, SGPT and ALP produced by CCl_4 indicating that *Polygala chinensis* whole plant extract protects liver injury induced by CCl_4 towards normalization. Silymarin, a prototype hepatoprotective agent also showed similar changes.

Bilirubin is the main bile pigment that is form the breakdown of heme in the red blood cells. It is transported to the liver where it is secreted by the liver into the bile. Conjugation of bilirubin is a prerequisite for its excretion into the bile [21]. Malfunctioning of the liver was evidenced by the significant increase (p< 0.01) in the level of unconjugated bilirubin in the serum of the group treated with only CCl_4 when compared to normal control. Increase in the level of unconjugated bilirubin in the blood may result from a defect in the function of the liver to conjugate the bilirubin being produced. The significant reduction (p< 0.05) of unconjugated bilirubin level in the serum when CCl_4 was simultaneously administrated with the

ethanol extract of *P. chinensis* when compared with the administration of CCl_4 alone indicates that the conjugating function of the liver was improved. The reduction of the unconjugated bilirubin level by the ethanol extract suggest that the extracts may activate the constitutive androstance receptor (CAR) which is a key regulator in bilirubin clearance in the liver [22]. The primary function of CAR is the bilirubin clearance pathway is to direct cooridnate response to elevated levels of bilirubin by increasing the hepatic expressive of each component of the pathway [23].

The ability of simultaneous administration of CCl_4 with ethanol extract of *P. chinensis* to significantly reduce ($p< 0.01$) the level of serum total bilirubin when compared with that of the CCl_4 treated group suggests the potential of the extract is clearing bilirubin from the serum when its level elevated. Since the results obtained for the serum total protein and albumin concentrations followed the same trend, it thus implicated the same mechanism by which the ethanol extract of *P. chinensis* exerts its effect on these parameters. The administration of CCl_4 alone may adversely interfere with protein metabolism probably by inhibiting the synthesis of proteins. Administration of ethanol extract of *P. chinensis* whole plant reversed these changes may be by increasing protein synthesis. This indicates the hepatoprotective activity of *P. chinensis* whole plant against damage by CCl_4. Stimulation of protein synthesis has been advanced as a contributory hepatoprotective mechanism which accelerates regeneration of cells[24].

Intake of CCl_4 results in excessive generation of free radicals. Free radicals are the reactive oxygen species (ROS) which are known to cause oxidative damage to number of molecule in cell, including membrane- lipids, proteins and nucleic acids [25]. In the present study the hepatic cellular injury might be due to increased oxidative stress and thereby leading to lipid peroxidation. The level of lipid peroxidation in the CCl_4 treated rats was assessed by measuring the levels of TBARS in the liver tissues [26].

The increased TBARS levels in the liver of CCl_4 treated animals indicate enhanced lipid peroxidation leading to tissue injury. The cellular antioxidant defense mechanism, which includes scavenging activities of enzymes viz., SOD, CAT and GPx plays an important role in scavenging toxic intermediates of reactive oxygen species. During hepatotoxicity these enzymes might be functionally impaired due to excess generation of free radicals creating oxidative imbalance.

SOD is metalloprotein catalyzing the dismutation of superoxide anion to hydrogen and oxygen. Numerous studies have shown the importance of SOD in protecting cells against oxidative stress [27]. The SOD activity could be decreased in tissue during CCl_4 injection. This decrease could be due to the feedback inhibition or oxidative inactivation of enzyme protein due to excess ROS generation [28].

CAT, hemeprotein, catalyzes the reduction of hydrogen peroxides [29], acts as preventive antioxidant and plays an important role in protection against the deleterious effects of lipid peroxidation [30].The activity levels of catalase in tissue decreased in CCl_4 treated animals might be due to the inhibition of CAT activity, which is suggestive to enhanced synthesis of O_2^- is a powerful inhibitor of catalase [31].

GPx is an enzyme with selenium in the form of selenocysteine and can catalyze the reduction of hydrogen peroxide and hydroperoxidases to non toxic products: GPx has a well-established role in protecting cells against oxidative injury. GPx is non-specific for H_2O_2 and lipid peroxide generated during CCl_4 treatment which is efficiently scavenged by GPx activity. The depression of this enzyme activity reflects perturbations in normal oxidative mechanism during CCl_4 treatment.

The cellular antioxidant defense enzymes viz., SOD, CAT and GPx were significantly reduced in the CCl_4 treated rats. This might lead to decreased antioxidant defense and increased oxidative stress and thereby the tissue injury occurs. Similar studies also indicate the failure of cellular antioxidant defense system during hepatotoxicity was recorded [32, 33].

In conclusion, the results of this study demonstrate that the ethanol extract of *Polygala chinensis* whole plant have a potent hepatoprotective action aqueous CCl_4 induced hepatic damage in rats. Its mode in affording the hepatoprotective activity against CCl_4 induced liver damage may be due to cell membrane stabilization, hepatic cells regeneration and enhancement of antioxidant enzymes such as catalase, superoxide dismutase and glutathione peroxidase production. The hepatoprotective and antioxidant potential of whole plant extract could have been brought about by various phytochemical principles i.e. flavonoids, alkaloids, phenolics and tannins present in *P. chinensis* whole plant. So results of this study demonstrated that the *P. chinensis* has significantly protection on CCl_4 induced hepatotoxicity.

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References

1. Subramonium A, Pushpangadan P. Development of phytomedicines for liver diseases. *Inter. J. Pharmacol* 1999; 31: 166-175.
2. Lohiya NK. Plant products for contraception: How to make it a reality? In: Puri CP (Edn), ISSRF News letter. Vol 5. Indian Society for the study of reproduction and fertility. Mumbai pp 9-12.
3. Guffin M. Mc, Hobbs C, Upton R. (eds): American Herbal Products Associaton Botanical Safety Handbook. Boca Raton, FL: CRC Press, 89, (1997).
4. Alagammal M, Rajalakshmi K, Mohan VR. Antidiabetic and antihyperlipidiaemic activity of *Polygala chinensis* L. whole plant in alloxan induced diabetic rats. *International Journal of Chemical and Pharmaceutical Sciences* 2012; 3: 37-44.
5. Alagammal M, Daffodil ED, Mohan VR. Anti-inflammatory activity of *Polygala chinensis* L. whole plant (Polygalaceae). *International Journal of Chemical and Pharmaceutical Sciences* 2012; 3: 19-21.
6. Rajalakshmi K, Mohan VR. Antioxidant properties of *Polygala chinensis* L. whole plant on alloxan induced diabetic rats. *International Journal of Pharmaceutical Sciences and Research* 2013; 4: 330-334.
7. Brinda P, Sasikala P, Purushothaman KK. Pharmacognostic studies on Merugankizhangu. Bulletin in Medical Ethnobotanical Research 1981; 3:84-96.
8. Anonymous. Phytochemical investigation of certain medicinal plants used in Ayurveda. Central Council for Research in Ayurveda and Siddha, New Delhi: 1990.
9. Lala PK. Lab manuals of Pharmacognosy CSI Publishers and Distributers, Kolkata: 1993.
10. OECD, (Organization for Economic co-operation and Development). OECD guidelines for the testing of chemicals/Section 4: Health Effects Test No. 423; Acute oral Toxicity- Acute Toxic Class method. OECD. Paris: 2002.
11. Lowry OH, Rosenbrough NJ, Farr AL, Randall RJ. Protein measurement with the Folin's phenol reagent. *Journal of Biological Chemistry* 1951; 193: 265-275.
12. Shanmugasundaram R, Kalpanadevi V, Tresina PS, Maruthupandian A, Mohan VR. Hepatoprotective activity of ethanol extracts of *Clitoria ternatia* L. and *Cassia angustifolia* Vahl. leaf against CCl_4 induced liver toxicity in rats. *Int Research J Pharmacy* 2010; 1: 201-205.
13. Thangakrishnakumari S, Nishanthini A, Muthukumarasamy S, Mohan VR. Hepatoprotective and antioxidant activity of *Canscora perfoliata*Lam (Gentiaceae) against CCl_4 induced hepatotoxicity I rats. *Int. J. Res. Ayur. Pharm* 2012; 3: 822-826.
14. Pal A, Banerjee B, Banerjee T, Masih M, Pal K. Hepatoprotective activity of *Chenopodium album* Linn. plant against Paracetamol induced hepatic injury in rats. *Int. J Pharm Sci* 2011; 3: 55-57.
15. Anitha M, Daffodil ED, Muthukumarasamy S, Mohan VR. Hepatoprotective and antioxidant activity of ethanol extract of *Cynoglossum zeylanicum* (Vahl ex Hornem) Thurnb ex Lehm in CCl_4 treated rats. *J Applied Pharmaceutical Science* 2012; 2: 099-6.
16. Suky TMG, Parthiban B, Kingston C, Tresinasoris P, Mohan VR. Hepatoprotective and antioxidant activity effect of *Balanites aegyptiaca*(L) Del against CCl_4 induced heptotoxicity in rats. *Int J Pharmaceutical science and Research* 2011; 2: 887-892.
17. ShanazBanu, Arunachalam G, Jajaveera KN, AshokaBabu VL, Vimal Kumar. Hepatoprotective activity of methanolic extract of *Barleria Montana* leaves in ethanol treated rats. *Asian Pac J Trop Disease* 2012; S748-S752.
18. Velayudam, Arul Amuthan, Ilavarasan. Hepatoprotective activity of Kadukkai Maathrai (A Siddhapolyherbal formulation) against CCl_4 induced liver damage in rat. *Research J Pharmaceutical Sciences* 2012; 1: 17-21.
19. Vipin Kumar, Pankaj K Modi, Saxena KK. Exploration of heptoprotective activity of aqueous extract of *Tinospora cordifolia*an experimental study. *Asian J Pharmaceutical and Clinical research* 2012; 6: 87-91.

20. Sheeba KO, Wills PJ, Latha BK, Rajalekshmy R, Latha MS. Antioxidant and antihepatotoxic efficacy of methanolic *extract* of *Elephantopus scaber* Linn in Wistar rats. *Asian Pac J Trop Disease 2*012; S904-S908.
21. Iniaghe OM, Malomo SO, Adebayo JO. Hepatoprotective effect of the aqueous extract of leaves of *Acalypha racemosa* in carbon tetrachloride treated rats. *Int J Med Plants Res* 2008; 2: 301-305.
22. Moore D, Jun Z, Werdorg H. Induction of bilirubin clearance by xenobiotic receptor CAR. *J Clinc Invest* 2004; 113: 137-143.
23. Gupta M, Mazumdar UK, Kumar RS, Sivakumar T, Gomathi P, Rajeshwar Y. Antioxidant defense system induced by a methanol extract of *Caesapinia bonducella* in rat liver. *Pharmaceutical Biology* 2005; 43: 411-419.
24. Rajalakshmi G, Arul Jothi K, Venkatesan RS, Jegatheesan K. Hepatoprotective activity of *Andrographis paniculata* on paracetamol induced liver damage in rats. *J Pharmacy Research* 2012; 5: 2983-2986.
25. Lobo V, Patil A, Phatak A, Chanda N. Free radicals, antioxidants and functional foods: Impact on human health. *Phcogrev* 2010; 2: 118-126.
26. Majnu V, Nalini N. Effect of ginger on lipid peroxidation and antioxidant status in 1,2-dimethyl hydrazine induced experimental colon carcinogenesis. *J Biochem Technol* 2010; 2: 161-167.
27. Yang ES, Lee JH, Park JW. Ethanol induces peroxynitrite-mediated toxicity through inactivation of $NADP^+$ - dependent isocitrate dehydrogenase and superoxide dismutase. *Biochimie* 2008; 90: 1316-1324.
28. Shanmugam KR, Ramakrishna CH, Mallikajurna K, Reddy KS. Protective effect of ginger against alcohol induced renal damage and antioxidant enzymes in male albino rats. *Indian J Exp. Biol* 2010; 48: 143-149.
29. Dash DK, Yeligar VC, Nayak SS, Ghosh T, Rajalingam D, Sengupla P. Evaluation of hepatoprotective and antioxidant activity of *Lchnocarpus frutescens*(Linn.) R.Br. on paracetamol-induced hepatotoxicity in rats. *Trop J Pharm Res* 2007; 6: 755-765.
30. Dinkova-Kostova AT. Protection against cancer by plant phenyl propenoids; induction of mammalian anticarcinogenic enzymes. *Mini Rev Med Chem* 2002; 2: 595-610.
31. Popovic M, Janicijevic-Hudomal S, Kaurinovic B, Rasic J, Trivic S. Effects of various drugs on alcohol induced oxidative stress in the liver. *Molecules* 2008; 13: 2249-2259.
32. Anitha M,Rajalakshmi K, Muthukumarasamy S, Mohan VR. Hepatoprotective and antioxidant activity of *Cynoglossum zeylanicum* (Vahl ex Hornem) Thurnb ex Lehm in CCl_4 treated rats. *J Applied Pharmaceu. Sci* 2012; 2: 099-103.
33. Thangakrishnakumari S, Nishanthini A, Muthukumarasamy S, Mohan VR. Hepatoprotective and antioxidant activity of *Canscora perfoliata* Lam. (Gentianaceae) against CCl_4 induced hepatotoxicity in rats. *International J Research in Ayurveda and Pharmacy* 2012; 3: 822-826.