African Journal of Biochemistry Research Vol. 4(8), pp.196-203, August 2010
Available online at http://www.academicjournals.org/AJBR
ISSN 1996-0778 ©2010 Academic Journals

Full Length Research Paper

In vitro and *in vivo* evaluation of free radical scavenging potential of ethanolic extract of *Podophyllum hexandrum*

Showkat Ahmad Ganie[1], Mohmmad Afzal Zargar[1], Akbar Masood[1] and Ehtishamul Haq[2]*

[1]Department of Biochemistry University of Kashmir, Srinagar, 190006, India.
[2]Department of Biotechnology, University of Kashmir, Srinagar, 190006, India.

Accepted 11 August, 2010

The study was aimed at evaluating the antioxidant activity of ethanolic extract of rhizome of *Podophyllum hexandrum* under *in vitro* and *in vivo* situations. The extract was found to contain a large amount of polyphenols and also exhibit an immense reducing ability. At a concentration of 250 µg/ml, 48% of H_2O_2 radicals could be scavenged by the extract. The extract also inhibited hydroxyl radical ($^{\bullet}OH$) induced oxidation of protein (BSA). The ethanolic rhizome extract of *P. hexandrum* also exhibited a significant antioxidant activity in acute oxidative tissue injury animal model constituted by CCl_4 induced hepatotoxicity. Oral administration of the extract at a dose of 20 and 50 mg/kg bw significantly protected from CCl_4 induced elevation in aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) in the serum, depletion of hepatic protein and GSH content, decrease in the activities of hepatic antioxidant enzymes: glutathione peroxidase (GPx), glutathione reductase and glutathione-S-transferase.

Key words: *Podophyllum hexandrum*, CCl_4, antioxidant activity, free radical scavenging.

INTRODUCTION

In biological systems the generation of free radicals is a normal phenomenon. Sometimes these free radicals are generated to such an extent that the body's defense mechanism is not able to remove them and as a result stress develops in the body. This process is called oxidetive stress and it is defined as a shift in the prooxidant-antioxidant balance towards a prooxidant environment. The free radicals are the chemical species which have unpaired electrons and are thus very unstable and reactive. In order to attain stability they react with their neighboring atoms to gain the electrons and as a result new free radicals are generated, which in turn attack the other nearby molecules causing a cascade of reactions (Okezie, 1996).

Aerobic organisms use oxygen to oxidize food and obtain the energy, a phenomenon essential for their sustenance. But during this oxidation process the oxygen molecule themselves get reduced and form intermediates called reactive oxygen species (ROS). The intermediates that are formed during the oxidizing process are hydrogen peroxide radicals ($H_2O_2^{\bullet}$) and hydroxyl radicals ($OH^{\bullet}$) and superoxide radicals (O_2^{-}) (McCord et al., 1971). Free radicals are also formed exogenously due to interplay of factors external to the body. Certain pesticides, drugs, hepatotoxins, sunlight, ionizing radiations and pollutants including cigarette smoke are some of the known exogenous factors. The free radicals generated in the body cause several types of damages including peroxidation of unsaturated fatty acids in membranes.

Mitochondria and microsomal membranes contain relatively large amounts of polyunsaturated fatty acids and

*Corresponding author. E-mail: haq@kashmiruniversity.net. Tel: +91-9906444123.

hence are susceptible to the damage (Fleischer et al., 1965). It is already known that the membrane lipids being rich in unsaturated fatty acids undergo peroxidation both *in vitro* and *in vivo* (Witting, 1965). Free radicals are also responsible for causing the denaturation of proteins, carbohydrates and nucleic acids and are intermediates in the induction of cardiovascular diseases, inflammatory conditions, autoimmune disorders and cancer pathology (Hogg, 1998; Pong, 2003). Free radicals are continuously formed in the body but the aerobic organisms employ a battery of defense mechanisms such as antioxidant enzymes glutathione reductase (GR), glutathione peroxidase (GPx), glutathione–S-transferase (GST), superoxide dismutase (SOD) and catalase (CAT) to prevent or mitigate oxidative tissue damage (Halliwell et al., 1989). When the liver cell plasma membrane is damaged, many of the enzymes normally located in the liver cell cytosol are released into the blood stream. Their estimation in the blood is a useful quantitative marker of the extent and type of hepatocellular damage (Mitra et al., 1998). In addition, perturbation of the GSH status of a biological system has been reported to increase the lipid peroxidation (Uday et al., 1999). Thiobarbituric acid reactive substances, TBARS, are produced as by-products of lipid peroxidation that occurs in the hydrophobic core of biomembranes (Fraga et al., 1987). At other sites, intake of compounds that induce antioxidant enzyme activity or scavenging of free radicals prevents oxidative damage (Hochstein et al., 1988). Antioxidants are the substances that when present at a low concentration compared with those of the oxidizable substances considerably delay or inhibit the oxidation of the substrate. Antioxidants act as a major defense against radical mediated toxicity by protecting the damage caused by free radicals (Nayan and Janardhanan, 2000). Further-more medicinal plants are used as 'antioxidants' in traditional medicine, their claimed therapeutic properties could be, in part, due to the presence of organic compounds being able to scavenge oxygen free radicals.

P. hexandrum belongs to the Berberidaceae family, it is locally known as Banwangon in the Kashmiri region of J and K, North India. It is a fibrous, juicy herb, 35 - 60 cm high with perennial rhizome (tubers) bearing numerous roots. *P. hexandrum* grows in the inner range of Himalayas from Kashmir to Sikkim (India) at an altitude of 3000 – 4200 m over sea level. The dried rhizomes and roots of this plant are traditionally used for medical purposes. Podophyllin a resin (derived from *P. hexandrum*) has cholagogue, purgative and emetic pro-perties. The rhizome powder is locally used as a laxative, to treat intestinal parasites (worms), warts and tumorous growths on the skin." Application of podophyllum resin 25% solution is efficacious in producing significant short-term resolution of HIV related oral hairy leukoplakia (Cobb, 1990; Gowdey et al., 1995). Podo-phyllatoxin isolated from *P. hexandrum* is a natural lignin that is currently being used as a precursor to semi-synthetic anti-cancer drugs like etoposide, teniposide and etopophos. These compounds have been used for the treatment of lung and testicular cancers as well as certain leukemias (Stahelin and Wartburg, 1991; Imbert, 1998). Carbon tetrachloride (CCl_4) has been widely used in animal models to induce acute liver injury (Mizuoka et al., 1999; Rao et al., 1997; Czaja et al., 1995). It is generally believed that the toxicity of CCl_4 results from its reductive dehalogenation by the cytochrome P_{450} enzyme system into the highly reactive free radical trichloromethyl radical (Recknagel et al., 1989). The present study was carried out to examine free radical scavenging properties of an ethanolic rhizome extract of *P. hexandrum* and its protective effect in an animal model of hepatotoxicity induced by carbon tetrachloride.

MATERIAL AND METHODS

Plant materials and preparation of plant extract

The rhizome of *P. hexandrum* were collected from the higher reaches of Aharbal (J and K, North India) during the months of May - June of 2006 and authenticated by Dr. Irshad Nawchoo, Associate Professor of the Department of Botany, University of Kashmir Srinagar, India. A voucher specimen with herbarium number (KASH- bot/Ku/*PH*- 702- SAG) has been retained for future reference at the Taxonomy Centre of the University of Kashmir. The rhizome material was shade dried at 30 ± 2°C. The dried material was grinded to powder using mortar and pestle and sieved with a sieve of 0.3 mm aperture size. The powder obtained was successively extracted in hexane, ethyl acetate, absolute ethanol, 70% ethanol by using soxhlet extractor (Borosil New Delhi India) (60 - 80°C). The 70% ethanolic extract was then concentrated with the help of rotary evaporator under reduced pressure and the dried extract was stored at 4°C until further use.

Animals

Wistar male albino rats, weighing 200 – 250 g, were purchased from the Indian Institute of Integrative Medicine (IIIM), Jammu J and K India. The animals were fed on a pellet diet (Hindustan Lever, Ltd., Mumbai, India) and water *ad libitum*. The animals were maintained in a controlled environment under standard conditions of temperature and humidity with an alternating 12 h light and dark cycle, in accordance with the guidelines prescribed by the National Institute of Nutrition, Indian Council of Medical Research. The use of animals for the current study was duly approved by the Animal Ethic Committee of the University of Kashmir.

Determination of phenolic content

The total phenolics in 70% ethanolic extracts of *P. hexandrum*

rhizome were determined by using Folin- Ciocalteau reagent according to the protocol of (Chandler et al., 1993). Quantitation was based on the standard curve of gallic acid (10 mg%), which was dissolved in methanol/water (60:40, v/v, 0.3% HCl). The concentration of polyphenols was expressed in terms of mg/100 ml of sample.

Reducing power

The reducing power of *P. hexandrum* rhizome extract was evaluated according to Oyaizu (1986). Different concentrations of the plant extracts were mixed with 2.5 ml of 0.2 M phosphate buffer (pH 6.6), and 2.5 ml of 1% potassium hexacyanoferrate (11). The mixture was incubated at 50°C for 20 minutes, 2.5 ml of 10% TCA was added to the mixture and centrifuged at 3000rpm for 10 minutes. The upper layer of the solution (2.5ml) was mixed with distilled water (2.5ml) and $FeCl_3$ (0.5 ml, 0.1%), and the absorbance was measured at 700nm by using spectrophotometer. Butylated hydroxyltolune (BHT; Sigma Aldrich) was used as standard.

Protein oxidation

Bovine serum albumin (BSA) was oxidized by a Fenton- type reaction. BSA (1 mg/ml) was incubated at 25°C in solution with 2.5 mM H_2O_2, 1.0 mM ascorbic acid and 3.0 mM EDTA in the presence or absence of 70% ethanolic rhizome extract of *P. hexandrum*. After incubation for 45 min, protein was precipitated with 10% TCA, centrifuged at 5000 rpm (Remi C-24 India), at 4°C for 10 min and the supernatant was decanted, protein pellets were dissolved in minimum amount of 50 mM potassium phosphate buffer, pH 7.5. Total sulphydryl (-SH) group determinations were performed according to the method of Moren et al. (1979) using Ellman's reagent (DTNB). The absorbance was measured at 412 nm spectrophotometrically (Elico India), and total sulphydryl groups were expressed as nmoles per mg protein using 5, 5- Dithiobis (2-nitrobenzoic acid) (DTNB) molar extinction coefficient of 13,100.

Assessment of hydrogen peroxide scavenging activity

The ability of *P. hexandrum* extracts to scavenge hydrogen peroxide was evaluated according to the method of Ruch et al. (1989). A solution of H_2O_2 (2 mmole) was prepared in phosphate buffer (pH = 7.5). Plant extract (50 – 250 µg/ml) were added to the hydrogen peroxide solution (0.6 ml). Absorbance of hydrogen peroxide at 230 nm was determined after 15 min against a blank solution containing only phosphate buffer without hydrogen peroxide. BHT was used as standard.

Biochemical analysis

Rats were divided into six groups containing seven animals each. The plant extract was employed at oral doses of 20 and 50 mg/kg body wt./day. The extract was suspended in normal saline such that the final volume of extract at each dose was 1 ml and was given to rats through gavage.

Treatment groups

Group I- Received olive oil only (vehicle) 5.0 ml/kg and served as normal control. Group II- Received only CCl_4 1.0 ml/Kg body weight (suspended in olive oil). Group III- Animals were administrated with Vitamin E (α-tocopherol; Sigma Aldrich) 50 mg/Kg body weight.

Group IV- Received 20 mg/Kg body weight of *Podophyllum* extract orally for all fifteen days. Group V- Received 50 mg/kg body weight plant extract orally for all fifteen days. On the thirteenth day, animals in the groups II - V were treated with CCl_4 at the dose of (1 ml/Kg body weight) by means of an intraperitoneal injection. After 24 h of CCl_4 administration (that is, 15th day), the rats were sacrificed and liver tissue was isolated and post mitochondrial supernatant (PMS) were prepared. The blood was collected from retro-orbital plexus without the use of anticoagulant. The blood was allowed to stand for 10 min at room temperature before being centrifuged at 2,000 rpm for 10 min to obtain serum for analysis of alanine aminotransferase (ALT), aspartate aminotransferase (AST) and serum lactate dehydrogenase (LDH).

Enzyme activity estimation

The estimation of enzymes activities were performed as indicated in the respective references: ALT and AST activity were carried out by the method of (Reitman and Frankel, 1975), LDH by (King, 1965), Glutathione peroxidase (Lawrence et al., 1976), Glutathione reductase (Mannervik et al., 1985), Glutathione-S-transferase (Habig et al., 1974), GSH (Hissin et al., 1976). Protein estimation was done by the method of (Lowry et al., 1951).

Statistical analysis

The values are expressed as mean ± S.D. The results were analyzed statistically by using Graphpad Prism 5 software using one-way ANOVA followed by Bonferroni t-test.

RESULTS

The yield of extract obtained from *P. hexandrum* rhizome using 70% ethanol as solvent was found to be 30% (w/w). The extract was found to contain 153 ± 12 mg/g, total phenolics expressed as gallic acid equivalent (mg/g of gallic acid). Since polyphenols are responsible for the antioxidant activity, the obtained amount of total polyphenols in the extract indicates the extract to possess a high antioxidant activity.

Reducing power is a measure of reductive ability of antioxidants and is evaluated by the transformation of Fe^{3+} to Fe^{2+} in the presence of sample extract. The reducing capacity of a compound may serve as a significant indicator of its potential antioxidant activity. The reducing power of 70% ethanolic extract of *P. hexandrum* is shown in Figure 1. The data shows that the reducing power of extract increased in a dose dependent manner. The ability of reducing power of 70% ethanolic extract was almost comparable with the synthetic antioxidant, butylated hydroxyl toluene (BHT). At a high concentration of 500 µg/ml, the reducing power of 70% ethanolic extract was found to be 77% as compared to 98% inhibition seen

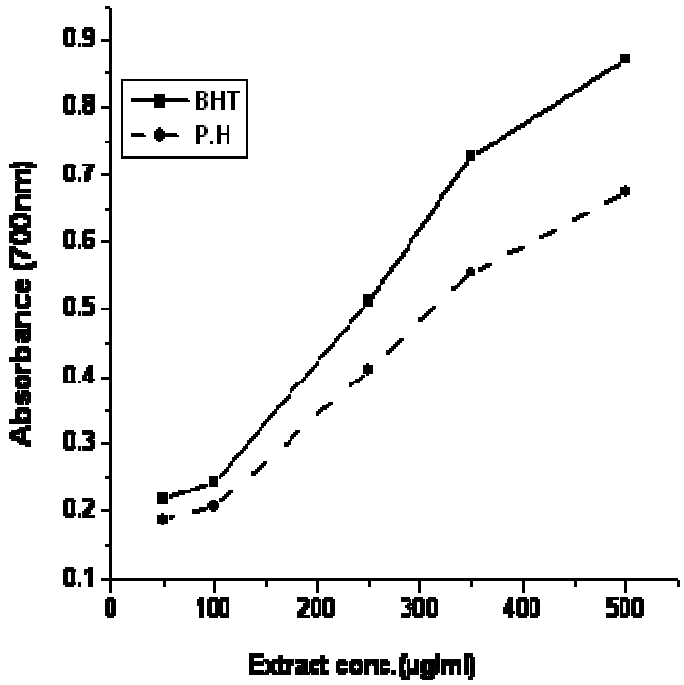

Figure 1. Reducing power (Fe^{3+} to Fe^{2+} conversion) shown by ethanolic extract of *P. hexandrum* and known antioxidant BHT; Butylated hydroxyltolune. IC_{50} values BHT=187, E.E = 266. The data are presented as means ± S.D of three independent experiments. E.E; ethanolic extract of *P. hexandrum*.

with BHT. The 50% inhibition of plant extract was found at a concentration of 266 µg/ml as compared to that of BHT that was 187 µg/ml (Figure 1).

Ethanolic extract of *P. hexandrum* effectively inhibited BSA oxidation induced by Fe- ascorbate- H_2O_2 system that generates $OH^{\bullet}$ radicals. BSA oxidation was determined in terms of – SH group loss by Ellman reagent (5, 5- dithiobis- (2–nitrobenzoic acid). The incubation of BSA with Fe- ascorbate- H_2O_2 complex caused the oxidation of 62% of the –SH groups, in the absence of any extract. The ethanolic extract of *P. hexandrum* rhizome dose dependently reversed this –SH oxidation (Figure 2). As can be seen in Figure 2, the 500 µg/ml extract was able to revert this oxidation to 36%, which was a significant change as compared to Fe-ascorbate- H_2O_2 complex.

The ability of *P. hexandrum* extracts to scavenge hydrogen peroxide is represented in Figure 3. This was assessed by taking hydrogen peroxide, under *in vitro* conditions and then adding increasing concentrations of *P. hexandrum* extract. The absorbance was taken at 230 nm, this is where H_2O_2 absorbs maximally and it was seen that the plant extract was effective in scavenging H_2O_2 in a concentration dependent manner as was evident by the decrease in absorbance. BHT which was used as a positive control also scavenged H_2O_2 in concentration dependent manner (Figure 3).

Studies using an animal model of hepatotoxicity

Effect on liver marker enzymes

The effect of *P. hexandrum* on serum marker enzymes is presented in Table 1. The levels of serum AST, ALT and LDH after 24 h of CCl_4 administration were markedly elevated in CCl_4 treated animals, indicating liver damage. Adminstration of *P. hexandrum* extract protected the livers from CCl_4 damage. The effect by 50 mg/Kg bw of the extract was similar to that provided in the presence of vitamin E, as no significant difference existed between these two groups, in the activities of ALT, AST and LDH (Table 1).

Enzymes involved in maintaining redox

The effect of *P. hexandrum* treatment on maintaining the machinery involved in maintaining redox is shown in Table 2. The damage to liver was also supported by decreased protein concentration in CCl_4 treated animals, again the level was restored by *P. hexandrum* treatment. Reduced glutathione content in liver homogenate was significantly ($P < 0.001$) decreased in CCl_4 treated animals in comparison to control group. The *P. hexandrum* treatment appreciably restored the GSH levels. Glutathione reductase (GR) and Glutathione peroxidase (GP_X) activity were significantly decreased ($P < 0.001$) in Group II CCl_4 treated rats in comparison to control. A dose dependent restoration of both GR and GPx activities was observed with increasing concentration of plant extract (Table 2). Similar trend was observed with Glutathione-S-transferase (GST). Treatment with vitamin E, a known antioxidant also significantly increased the above mentioned activities.

DISCUSSION

Free radicals formed in the body react with variety of biomolecules creating an undesirable situation known as oxidative stress, resulting ultimately in disturbances of prooxidant/antioxidant equilibrium status. The equilibrium could be restored back by supplementing with exogenous antioxidant and or activating endogenous antioxidant system. It has long been recognized that naturally occurring substances in plants have antioxidant activities.

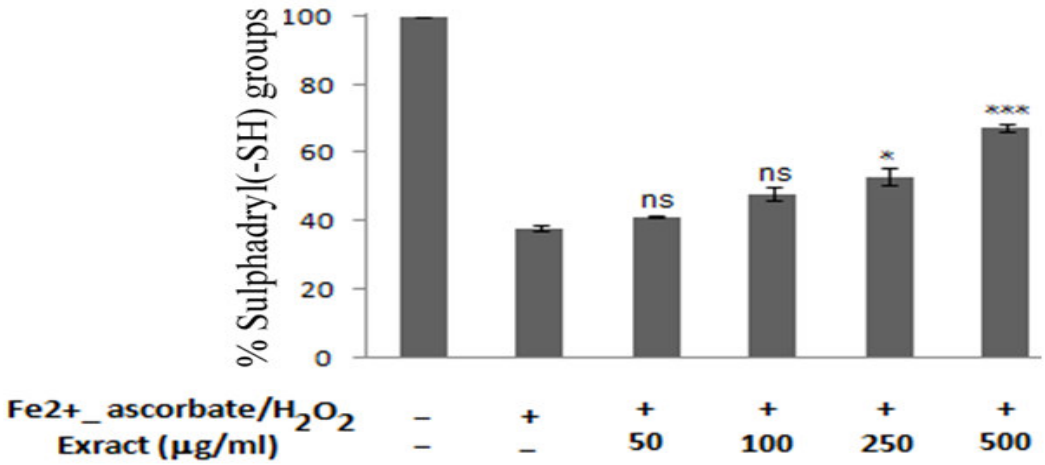

Figure 2. Inhibitory effect of ethanolic extract of *P. hexandrum*x on BSA oxidation induced by Fe^{2+}-ascorbate/ H_2O_2 system. Each value is the mean of three determinations. ***$P < 0.001$, *$P < 0.05$, ns-non significant as compared to without extract but with Fe2+ - ascorbate/H_2O_2.

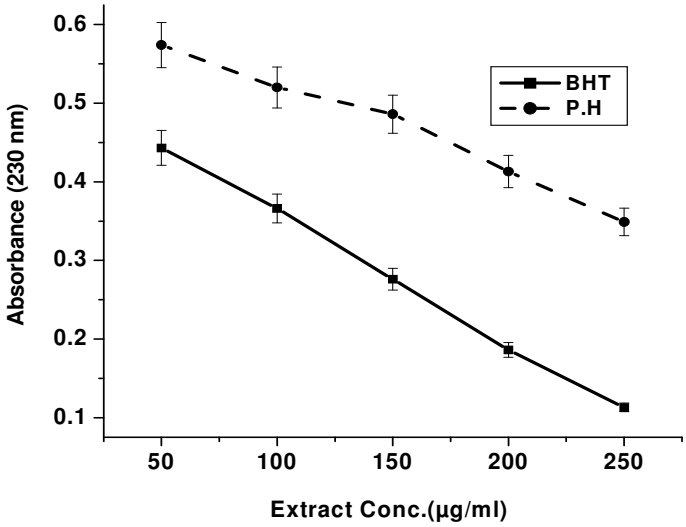

Figure 3. Hydrogen peroxide radical scavenging activity of ethanolic extract of Podophyllum hexandrum and known antioxidant BHT, Butylated hydroxyltolune. Absorbance of control = 0.671 ± 0.017, IC_{50} values BHT=109, E.E = 323.The data are presented as mean ± S.D of three independent experiments. E.E; ethanolic extract of *P. hexandrum*.

In the present study, we investigated the antioxidant potential of 70% ethanolic extract of *P. hexandrum.* Antioxidant activity of the plant extracts is often ascribed to the phenolic compounds present in them. Phytochemicals, especially plant phenolics constitute a major group of compounds that act as primary antioxidants (Hatano et al., 1989). The phenolic content of 70% ethanolic *P. hexandrum* rhizome extract was 153 ± 12 mg/g gallic acid. In various studies that evaluated the antioxidant activity of medicinal plants and fruits, a linear relationship between the total phenolic content and the antioxidant activity has been found indicating that the phenolic compounds might be the major contributors to the antioxidant activities of these extracts (Banerjee et al., 2005).

Glutathione is one of the most abundant tripeptide, non-enzymatic biological antioxidant present in liver. It removes free radicals species such as H_2O_2, $^{\bullet}O_2$ and maintains protein thiols. Is is a substrate for GPx also. Hydrogen peroxide can attack many cellular energy producing system e.g. glycolytic enzymes. It has been reported that removal of hydrogen peroxide as well superoxide anion is very important for protection of pharmaceutical and food systems (Gulcin et al., 2004). The extract used during this study, also scavenged H_2O_2, and also restored the levels of antioxidant enzymes viz., GPx, GR and the level of GSH (reduced), which had otherwise been significantly decreased in Group II CCl_4 treated rats (Table 2). Similar findings were reported by Palanivel et al. (2008) for *Pisonia aculeate L.* against CCl4 induced hepatic damage in rats. The sulphydryl (SH) groups of proteins are crucial for several important functions. They maintain the functional conformation of proteins and also participate in catalytic activity of several enzymes. –SH groups, due to their ability to be reversibly oxidized, are recognized as key components involved in the mainte-nance of redox balance. 70% ethanolic extract of *P. hexandrum* showed appreciable protection against protein oxidation. Only few of plant extracts are reported to have this property (Martinez et al., 2001), pointing to the fact that the extract used during this study may have future beneficial applications in food and pharmaceutical

Table 1. Effect of ethanolic extract of *Podophyllum hexandrum* on serum hepatic markers in CCl4 induced hepatotoxicity in albino rats.

Treatment	Dosage	ALT (U/L)	AST (U/L)	LDH (U/L)
I. Control group (Olive oil only)	5 ml/Kg bw	20 ± 4	32 ± 12	46 ± 8
II. CCl_4 treated group	1 ml/Kg bw	137 ± 2***	115 ± 8***	253± 13***
III. CCl_4 treated + vitamin E	50 mg/Kg bw	76 ± 5***$$$	67 ± 7 ***$$$	181 ± 5***$$$
IV. CCl_4 treated + *P. H* extract	20 mg/Kg bw	104 ± 1***	105 ± 7***	224 ± 4***
V. CCl_4 treated + *P. H* extract	50 mg/Kg bw	87 ± 3 NS	75 ± 4 NS	193 ± 6 NS

$$$; p < 0 .001 compared to CCl_4,; ***p < 0 .001 compared to control group, NS-non significant when compared to CCl_4 + vitamin E. The data were presented as means ± S.D of six parallel measures and evaluated by one way ANOVA followed by the Bonferroni t – test. bw: body weight; ALT :alanine aminotransferase; AST: aspartate aminotransferase; LDH: lactate dehydrogenase; P.H: *Podophyllum hexandrum*.

Table 2. Effect of 70% ethanolic extract of *P. hexandrum* on antioxidant enzymes and protein levels of liver tissue in CCl_4 treated rats.

Parameters	Group I (Olive oil only)	Group II CCl_4 treated	Group III CCl_4 + V.E	Group IV 20 mg/kg *P. hexandrum* extract	Group V 50 mg/kg *P. hexandrum* extract
Protein Content (mg/100 mg tissue)	51.2 ± 0.2	12 ± 0.8	34.1 ± 0.4	13 ± 1.	27 ± 1.
Glutathione Reduced (nm/g protein)	128 ± 21	34 ± 0.7***	93 ± 8$$$	60 ± 3***	70 ± 2#
Glutathione reductase (µg GSSG utilized/minute/mg protein)	51 ± 20	2.6 ± 0.7***	42 ± 10$$$ns	8 ± 0.8***	16 ± 1###
Glutathione peroxidase (µg GSH utilized/minute/mg protein)	50 ± 9	5.9± 0.7***	42 ± 6 $$$ns	7.4 ± 0.6***	19 ± 0##
Glutathione- S- transferase (nmoles of CDNB conjugated/min/mg protein)	44 ± 1	11.4 ± 0.5***	39 ± 1$$$*	18 ± 2***	34 ± 2#

* $p < 0.05$, ** $p < 0.001$, ns-nonsignificant as compared to normal group, #p,0.05, ## p < 0-.01, ###$p < 0.001$as compared with CCl_4 +V.E, $$$ p < 0.001 to CCl_4 induced group. The data represent the means ± SD for 7 animals in each group and evaluated by one-way ANOVA following Bonferroni t-test. Differences were considered to be statistically significant if $p < 0.05$. V.E: Vitamin E. P.H, *P. hexandrum*.

industry.

The reductive capacity of the extract was further studied using the Fe^{3+} to Fe^{2+} reduction assay. In this method, polyphenols reduce ferrocyanide ions i.e. $[Fe(CN)_6]^{3-}$ to ferric ferrocyanide $Fe_4[Fe(CN)_6]_3$ which in turn give rise to Prussian blue coloured complex. It is a known fact that phenolic acids and phenols having more number of reactive OH groups attached to ring act as more powerful reducing agents. This ultimately results in the neutralization of free radical chain reactions. Thus the most powerful reducing agents are in general the most efficient radical scavengers (Andersen et al., 2003). Our results suggest that polyphenolic components within the 70% ethanolic

extract of *P. hexandrum* may play a critical role in radical scavenging properties under *in vitro* and *in vivo* situations. Similar results were reported by Wang et al., (2009) for marine red alga, *Rhodomel confervoides* with high relationship between phenolic compounds and reducing power of the extract.

CCl_4 administration to rats causes changes in the serum levels of marker enzymes for liver damage and hepatic antioxidant enzymes, which is due to the CCl_4 damage to the liver parenchymal cells (Singh, 1980).When there is hepatopathy, these enzymes leak into the blood stream in conformity with the extent of liver damage (Nkosi et al., 2005). Accordingly the assessment of the level of AST, ALT and LDH provides a good and simple tool to measure the protective activity of the target drug against the hepatic damage (Hewawasan et al., 2004). The elevated level of these marker enzymes observed in the group II CCl4 treated rats in this present study corresponded to the extensive liver damage induced by CCl_4. The reduced concentrations of ALT, AST and LDH as a result of plant extract administration observed during this study (Table 1), indicated hepato-protection. Similar results were reported by different authors, when they used different plant extracts to study CCl_4 induced hepatic damage (Samudram P et al., 2008; Palanivel et al., 2008; Lima et al., 2010). Our results demonstrate that P. *hexandrum* ethanolic extract treatment prevents CCl_4 induced hepatotoxicity in rats by strengthening the antioxidant defense system and by scavenging free radicals. Detailed studies are warranted to look for the active ingredient in the extract that could be of possible therapeutic use.

REFRENCES

Andersen ML, Lauridsen RK, Skibsted LH (2003). Optimizing the use of phenolic compounds in foods. In: Johson I, Williamson G, eds. phytochemical functional foods. Cambridge: Woodhead Publishing Ltd. 315-346.

Banerjee A, Dasgupta N, De B (2005). *In vitro* study of antioxidant activity of *Syzygium cumini* fruit. Food Chem. 90: 727-733.

Chandler SF, Dodds JH (1993). The effect of phosphate, nitrogen and sucrose on the production of phenolics and solasidine in callus cultures of *Solanum laciniatum*. Plant Cell Rep. 2: 1005-110.

Cobb MW (1990). Human Papilloma virus infection. J. Am. Acad. Dermatol., 22: 547.

Czaja MJ, Xu J, Alt E (1995). Prevention of carbon tetrachloride induced rat liver injury by soluble tumor necrosis factor receptor. Gastroenterol. 108: 849-54.

Fleicher S, Rouser G (1965). Lipid of subcellular particles. J. Am. oil chemists Soc. 42: 588.

Fraga C, Leibovitz B, Tappel A (1987). Halogenated compounds as inducers of lipid peroxidation in tissue slices. Free Rad. Biol. Med. 3: 119-123.

Gowdey G, Lee RK, Carpenter WM (1995). Treatment of HIV- related hairy leukoplakia with Podophyllum resin 25% solution. Oral Surg. Oral Med. Oral Pathol. Oral Radiol. Endocrinol., 79: 64.

Gulcin IG, Sat S, Beydemir SM, Elmasta, Kufrevioglu OI (2004). Food Chem. 87:393.

Habig VH, Pabst MJ, Jakoby WB (1974). Glutathione-S-transferase. The first enzymatic step in mercapturic acid formation. J. Biol. Chem. 249: 7130-7139.

Halliwell B, Gutteridge JMC (1989). Free Radicals in Biology and Medicine, 2nd ed. Oxford University Press. Oxford. pp. 78-93.

Hatano T, Edamatsu R, Mori A, Fujita Y, Yasuhara E (1989). Effects of tannins and related polyphenols on superoxide anion radical and on 1, 1-diphenyl-2-picrylhydrazyl. Chem. Pharm. Bull. 37: 2016-23.

Hewawasan RP, Jayatilaka KAPW, Pathirana C, Mudduwa LKW (2004). Hepatoproctective effect of *Epaltes divaricata* extract on carbon tetra chloride induced hepatotoxicity in mice. Ind. J. Med. Res. 120: 30-34.

Hissin PJ, Hilf R (1976). A fluorometric method for determination of oxidized and reduced glutathione in tissues. Anal. Biochem. 74: 214-226.

Hochstein P, Atallah AS (1988). The nature of oxidants and antioxidant systems in the inhibition of mutation and cancer. Mutation Res. 202: 363-37.

Hogg N (1998). Free radicals in disease. Seminars in reproductive endocrinology. 16: 241-288.

Imbert F (1998). Discovery of Podophyllatoxin. Bio.Chimie. 80: 207. *In vivo*. J. Am. Oil Chem. Soc. 42(11): 908.

King J (1965). The dehydrogenase of oxidoreductase- Lactate dehydrogenase. *In Practical Clinical Enzymology* (King JC and Van D, Eds.) Nostrand Co. Lundon. 83-93.

Lawrence RA, Burk RF (1976). Glutathione peroxidase activity in rat liver. Biochem. Biophys. Res. Commun. 71: 952-8.

Lima TB, Suja A, Jisa OS, Sathyanarayanan S, Remya KS (2010). Hepatoprotective activity of LIV-first against carbon tetra chloride-induced hepatotoxicity in albino rats. Int. J Green Pharm. 4(2): 71-74.

Lowry OH, Rosenbrough NJ, Farr AI, Randall RJ (1951). Protein estimation with the Folin-Phenol reagent. J. Biol. Chem. 193: 265-275.

Mannervik B, Carlberg I (1985). Glutathione Reductase. Methods in Enzymol. 113: 484-490.

Martinez G, Giuliani A, Leon OS, Perez G, Nunez, Selles AJ (2001). Effects of *Magnifera indica* L. extract (QF808) on protein and hepatic microsome peroxidation. Phytother. Res. 15: 581-585.

McCord JM, Keele BB, Fridovich I (1971). An enzyme base theory of obliate anaerobiosis: the physiological function of superoxide dismutase. PNAS. 68: 1024-1027.

Mitra SK, Venkataranganna MV, Sudaram R, Gopumadhavan S (1998). Protective effect of HD -03, a herbal formulation, against various hepatotoxic agents in rats. J. Ethnopharmacol. 63: 181-186.

Mizuoka H, Shikata N, Yang J, Takasu M, Inoue K, Tsubura, A (1999). Biphasic effect of colchicine on acute liver injury induced by carbon tetrachloride or by dimethylnitrosamine in mice. J. Hepatol. 1: 825-33.

Moren MA, Depierre JW, Mannervick B (1979). Levels of glutathione, glutathione reductase and glutathione S- transferase activities in rat lung and liver. Biochem. Biophys. Acta. 582: 67-78.

Nayan J, Janardhanan KK (2000). Antioxidant and antitumor activity of Pleurotus florida. Current Science. 79: 941-943.

Nkosi CZ, Opoku AR, Terblanche SE (2005). Effect of pumpkin seed (*Cucurbita pepo*) protein isolate on the activity levels of certain plasma enzymes in CCl4-induced liver injury in low-protein fed rats. Phy. Res. 19: 341-345.

Okezie IA (1996). Characterization of drugs as antioxidant prophylactic. Free radical Biology and Medicine. 20(5): 675-705.

Oyaizu M (1986). Studies on products of browning reaction prepared from glucosamine. Jap. J. Nutr. 44: 307-315.

Palanivel MG, Rajkapoor B, Kumar RS, Einstein JW, Kumar EP, Kumar MR, Kavitha K, Kumar MP, Jayakar B (2008). Hepatoprotective and Antioxidant Effect of *Pisonia aculeata* L. against CCl4- Induced Hepatic Damage in Rats Sci. Pharm., 76: 203-215.

Pong K (2003). Oxidative stress in neurodegenerative diseases: therapeutic implications for superoxide dismutase mimetics. Exp. Opin. Boil. Ther., 3: 127-139.

Rao PS, Mangipudy RS, Mehendale HM (1997). Tissue injury and repair as parallel and opposing responses to CCl4 hepatotoxicity: a novel dose-response. Toxicology., 118: 181-93.
Recknagel RO, Glende EA, Dolak JA, Waller RL (1989). Mechanisms of carbon tetrachloride toxicity. Pharmacol Ther. 43:139-54.
Reitman S, Frankel AS (1975). A Colorimetric method for the determination of serum glutamic oxaloacetic and glutamic pyruvic transaminases. Am. J. Clin. Pathol., 28: 56-63.
Ruch RJ, Cheng SJ, Klaunig JE (1989). Carcinogenesis. 10: 1003-1008.
Samudram P, Rajeshwari H, Vasuki R, Geetha A, Sathiya-moorthi P (2008). Hepatoprotective activity of Bi - herbal ethanolic extract on CCl_4 induced hepatic damage in rats. Afr. J. Biochem. Res. 2(2): 61-65.
Singh ID (1980). In: Text book of Biochemistry and Human biology (Talwar, G.P., ed), Prentice Hall of India. New Delhi
Stahelin HF, Wartburg AV (1991). The chemical and biological route form Podophyllatoxin glucoside to etoposide. Cancer Res. 51: 5.
Uday B, Das D, Banerjee KR (1999). Reactive oxygen species. Oxidative damage and pathogenesis, Curr. Sci. 77: 658-666.
Wang BW, Zhang Z, Duan, Li X (2009). In vitro antioxidative activities of extract and semi-purified fractions of the marine red alga, *Rhodomela confervoides* (Rhodomelaceae). Food Chem. 113: 1101-1105.
Witting LA (1965). Lipid peroxidation in vivo. J. Am. Oil Chem. Soc. 42 (11): 908.