

Full Length Research Paper

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

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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<sub>2</sub>O<sub>2</sub> radicals could be scavenged by the extract. The extract also inhibited hydroxyl radical (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<sub>4</sub> induced hepatotoxicity. Oral administration of the extract at a dose of 20 and 50 mg/kg bw significantly protected from CCl<sub>4</sub> 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<sub>4</sub>, 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 oxidative 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<sub>2</sub>O<sub>2</sub>) and hydroxyl radicals (OH) and superoxide radicals (O<sub>2</sub><sup>-</sup>) (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

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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 Banwangan 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 properties. 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<sub>4</sub>) 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<sub>4</sub> results from its reductive dehalogenation by the cytochrome P<sub>450</sub> 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- Ciocalteu 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<sub>3</sub> (0.5 ml, 0.1%), and the absorbance was measured at 700nm by using spectrophotometer. Butylated hydroxytoluene (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<sub>2</sub>O<sub>2</sub>, 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 sulphhydryl (-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 sulphhydryl 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<sub>2</sub>O<sub>2</sub> (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<sub>4</sub> 1.0 ml/Kg body weight (suspended in olive oil). Group III- Animals were administrated with Vitamin E ( -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<sub>4</sub> at the dose of (1 ml/Kg body weight) by means of an intraperitoneal injection. After 24 h of CCl<sub>4</sub> 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).

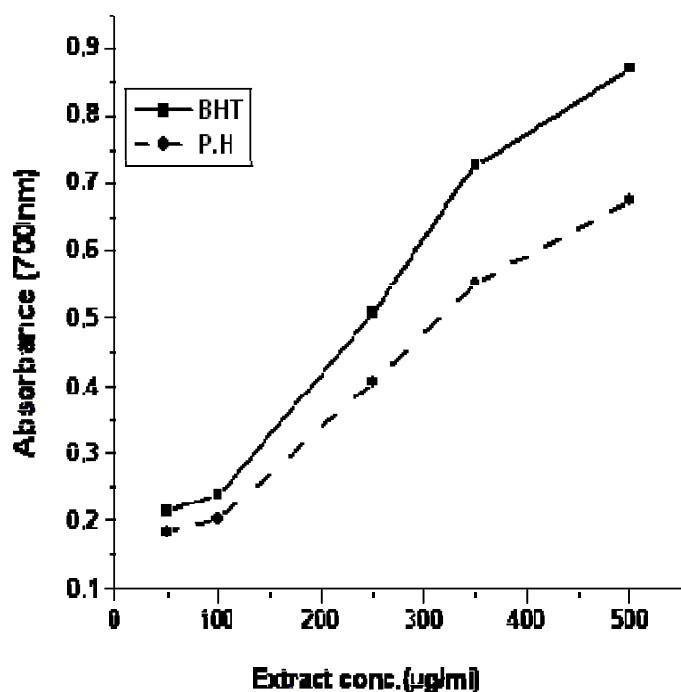
### Statistical analysis

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<sup>3+</sup> to Fe<sup>2+</sup> 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 with BHT.



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

The 50% inhibition of plant extract was found at a concentration of 266  $\mu\text{g/ml}$  as compared to that of BHT that was 187  $\mu\text{g/ml}$  (Figure 1).

Ethanolic extract of *P. hexandrum* effectively inhibited BSA oxidation induced by Fe- ascorbate-  $\text{H}_2\text{O}_2$  system that generates OH 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-  $\text{H}_2\text{O}_2$  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  $\mu\text{g/ml}$  extract was able to revert this oxidation to 36%, which was a significant change as compared to Fe- ascorbate-  $\text{H}_2\text{O}_2$  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  $\text{H}_2\text{O}_2$  absorbs maximally and it was seen that the plant extract was effective in scavenging  $\text{H}_2\text{O}_2$  in a concentration dependent manner as was

evident by the decrease in absorbance. BHT which was used as a positive control also scavenged  $\text{H}_2\text{O}_2$  in concentration dependent manner (Figure 3).

## Studies using an animal model of hepatotoxicity

### Effect on liver marker enzymes

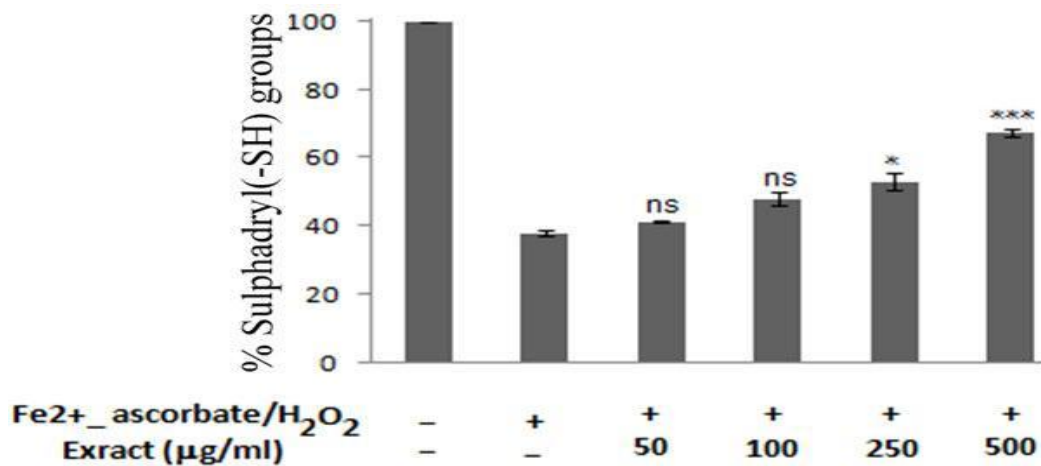
The effect of *P. hexandrum* on serum marker enzymes is presented in Table 1. The levels of serum AST, ALT and LDH after 24 h of  $\text{CCl}_4$  administration were markedly elevated in  $\text{CCl}_4$  treated animals, indicating liver damage. Administration of *P. hexandrum* extract protected the livers from  $\text{CCl}_4$  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

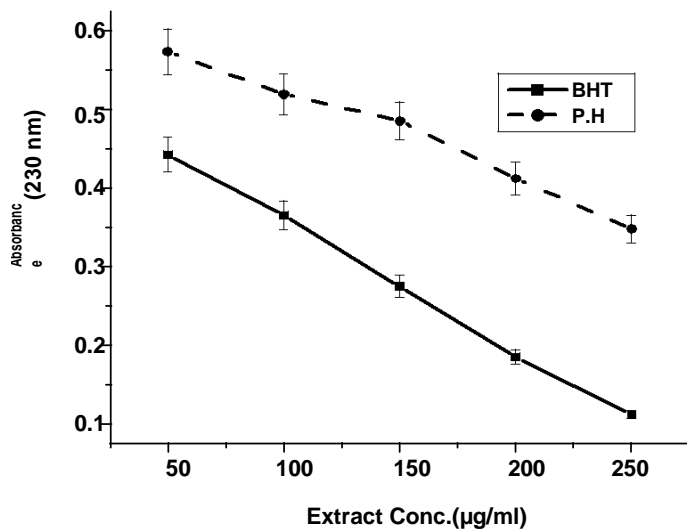
The effect of *P. hexandrum* treatment on maintaining the machinery involved in maintaining redox is shown in Table 2. The damage to liver was also supported by decreased protein concentration in  $\text{CCl}_4$  treated animals, again the level was restored by *P. hexandrum* treatment. Reduced glutathione content in liver homogenate was significantly ( $P < 0.001$ ) decreased in  $\text{CCl}_4$  treated animals in comparison to control group. The *P. hexandrum* treatment appreciably restored the GSH levels. Glutathione reductase (GR) and Glutathione peroxidase ( $\text{GPx}$ ) activity were significantly decreased ( $P < 0.001$ ) in Group II  $\text{CCl}_4$  treated rats in comparison to control. A dose dependent restoration of both GR and  $\text{GPx}$  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* on BSA oxidation induced by Fe<sup>2+</sup>-ascorbate/ H<sub>2</sub> O<sub>2</sub> 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 Fe<sup>2+</sup> - ascorbate/H<sub>2</sub>O<sub>2</sub>.



**Figure 3.** Hydrogen peroxide radical scavenging activity of ethanolic extract of *Podophyllum hexandrum* and known antioxidant BHT, Butylated hydroxytoluene. Absorbance of control = 0.671 ± 0.017, IC<sub>50</sub> 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<sub>2</sub>O<sub>2</sub>, O<sub>2</sub> and maintains protein thiols. It 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<sub>2</sub>O<sub>2</sub>, 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<sub>4</sub> treated rats (Table 2). Similar findings were reported by Palanivel et al. (2008) for *Pisonia aculeate* L. against CCl<sub>4</sub> induced hepatic damage in rats. The sulphhydryl (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 maintenance 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 CCl<sub>4</sub> 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 <sub>4</sub> treated group	1 ml/Kg bw	137 ± 2 <sup>***</sup>	115 ± 8 <sup>***</sup>	253 ± 13 <sup>***</sup>
III. CCl <sub>4</sub> treated + vitamin E	50 mg/Kg bw	76 ± 5 <sup>***\$\$\$</sup>	67 ± 7 <sup>***\$\$\$</sup>	181 ± 5 <sup>***\$\$\$</sup>
IV. CCl <sub>4</sub> treated + <i>P. H</i> extract	20 mg/Kg bw	104 ± 1 <sup>***</sup>	105 ± 7 <sup>***</sup>	224 ± 4 <sup>***</sup>
V. CCl <sub>4</sub> treated + <i>P. H</i> extract	50 mg/Kg bw	87 ± 3 <sup>NS</sup>	75 ± 4 <sup>NS</sup>	193 ± 6 <sup>NS</sup>

\$\$\$; p < 0.001 compared to CCl<sub>4</sub>; \*\*\*p < 0.001 compared to control group, NS -non significant when compared to CCl<sub>4</sub> + 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<sub>4</sub> treated rats.

Parameters	Group I (Olive oil only)	Group II CCl <sub>4</sub> treated	Group III CCl <sub>4</sub> + V.E	Group IV 20 mg/kg <i>P. hexandrum</i> extract	Group V 50 mg/kg <i>P. hexandrum</i> 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 <sup>***</sup>	93 ± 8 <sup>\$\$\$</sup>	60 ± 3 <sup>***</sup>	70 ± 2 <sup>#</sup>
Glutathione reductase (µg GSSG utilized/minute/mg protein)	51 ± 20	2.6 ± 0.7 <sup>***</sup>	42 ± 10 <sup>\$\$\$ns</sup>	8 ± 0.8 <sup>***</sup>	16 ± 1 <sup>###</sup>
Glutathione peroxidase (µg GSH utilized/minute/mg protein)	50 ± 9	5.9 ± 0.7 <sup>***</sup>	42 ± 6 <sup>\$\$\$ns</sup>	7.4 ± 0.6 <sup>***</sup>	19 ± 0 <sup>##</sup>
Glutathione- S- transferase (nmoles of CDNB conjugated/min/mg protein)	44 ± 1	11.4 ± 0.5 <sup>***</sup>	39 ± 1 <sup>\$\$\$*</sup>	18 ± 2 <sup>***</sup>	34 ± 2 <sup>#</sup>

\* p < 0.05, \*\* p < 0.001, ns-nonsignificant as compared to normal group, #p,0.05, ## p < 0-.01, ###p < 0.001as compared with CCl<sub>4</sub> +V.E, \$\$\$ p < 0.001 to CCl<sub>4</sub> 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<sup>3+</sup> to Fe<sup>2+</sup> reduction assay. In this method, polyphenols reduce ferrocyanide ions i.e. [Fe (CN) <sub>6</sub>]<sup>3-</sup> to ferric ferrocyanide Fe<sub>4</sub> [Fe

(CN) <sub>6</sub>]<sub>3</sub> 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, *Rhodmel confervoides* with high relationship between phenolic compounds and reducing power of the extract.

CCl<sub>4</sub> 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<sub>4</sub> 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 CCl<sub>4</sub> treated rats in this present study corresponded to the extensive liver damage induced by CCl<sub>4</sub>. The reduced concentrations of ALT, AST and LDH as a result of plant extract administration observed during this study (Table 1), indicated hepatoprotection. Similar results were reported by different authors, when they used different plant extracts to study CCl<sub>4</sub> 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<sub>4</sub> 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.

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