

***Podophyllum Hexandrum* Rhizome Methanolic Extract Ameliorates Carbon Tetrachloride Induced Hepatotoxicity In Albino Rats**

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**Summary**

Although treatment with therapeutic agents may be one of the main causes of liver toxicity, there have been only limited attempts at thorough research efforts to seek solution. In this study antioxidant activity of methanol extract of rhizomes of *Podophyllum hexandrum* was investigated using carbon tetrachloride (CCl<sub>4</sub>) - intoxicated rat liver as the experimental model. This CCl<sub>4</sub> induced hepatotoxicity was found to be associated with oxidative damage. The hepatotoxic rats were administered with the extract for 15 days (daily, orally at the dose of 20, 30 and 50 mg per kg body weight). Administration of CCl<sub>4</sub> at 1.0 ml/kg body weight developed acute hepatic damage, as demonstrated by increased serum alanine aminotransferase (ALT), Serum aspartate aminotransaminase (AST) and Serum lactate dehydrogenase (LDH) activity. In addition lipid peroxidation (LPO) in CCl<sub>4</sub> intoxicated rats was evidenced by a marked increment in the levels of thiobarbituric acid reactive substances (TBARS), and also a distinct diminution in glutathione (GSH) content in the liver. The decreased activity of antioxidant enzymes, such as superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPX) and glutathione reductase (GR) in CCl<sub>4</sub> intoxicated rats, and its retrieval towards near normalcy in CCl<sub>4</sub> + methanolic extract administered rats revealed the efficacy of extract in combating oxidative stress due to hepatic damage. Elevated level of glutathione transferase (GTS) observed in hepatotoxic rats too showed signs of returning towards normalcy in extract co-administered animals, thus corroborating the antioxidant efficacy of methanolic extract of *Podophyllum hexandrum*.

**Key words:** Antioxidant enzymes, Carbon tetrachloride, *Podophyllum hexandrum*, lipid peroxidation.

**Introduction**

Exposure to various organic compounds including a number of environmental pollutants and drugs can cause cellular damages through metabolic activation of those compounds to highly reactive substances such as reactive oxygen species (ROS). Free radical induced lipid peroxidation is believed to be one of the major causes of cell membrane damage leading to a number of pathological situations [1-3]. Reports from different laboratory and other investigators have established that the industrial solvent, carbon tetrachloride (CCl<sub>4</sub>) is a potent environmental hepatotoxin [4-7]. A number of recent reports clearly demonstrated that in addition to hepatic problems, CCl<sub>4</sub> also causes disorders in kidneys, lungs, testis and brain as well as in blood by generating free radicals [8-11].

Reports from Perez et al, Ogeturk et al and Churchill et al suggested that exposure to this solvent causes acute and chronic renal injuries [12-14]. Extensive evidence demonstrated that  $\bullet\text{CCl}_3$  and  $\bullet\text{Cl}$  are formed as a result of the metabolic activation of  $\text{CCl}_4$ , which in turn, initiate lipid peroxidation process. A known potent antioxidant, vitamin E, could protect  $\text{CCl}_4$  induced liver injury indicating that oxidative stress is responsible for  $\text{CCl}_4$  induced hepatic disorder in this particular model [15-16]. Studies also showed that various herbal extracts could protect organs against  $\text{CCl}_4$  induced oxidative stress by altering the levels of increased lipid peroxidation, and enhancing the decreased activities of antioxidant enzymes like superoxide dismutase (SOD), catalase (CAT) and glutathione-S-transferase (GST) as well as enhanced the decreased level of the hepatic reduced glutathione (GSH) [17]. In the modern medicine, plants occupy a significant birth as raw materials for some important drug preparations [18-19]. Our Kashmir valley is well known for a plethora of medicinal plants which act as antiradicals and DNA cleavage protectors. These plants have also been considered to protect health, longevity, intelligence, immunosurveillance and body resistance against different infections and diseases. *Podophyllum hexandrum* belongs to family Berberidaceae and in Kashmir valley, it is locally known as Banwangan. It is an erect flabrous, succulent herb, 35- 60 cm high with perennial rhizome bearing numerous roots and is found in the inner range of Himalayas from Kashmir to Sikkim at an altitude of 3000 - 4200m. The dried rhizomes and roots of this plant form the source of medicinal activity. The rhizome powder is used as a laxative or to get rid of intestinal worms and also used as poultice to treat warts and tumorous growths on the skin. It has been shown that the rhizome of *Podophyllum hexandrum* contains several lignins which possess antitumor activity. Podophyllatoxin is most active cytotoxic natural product by acting inhibitor of microtubule assembly. These drugs can be used for lung cancer, testicular cancer, neuroblastoma, hepatoma and other tumors [20].

*Podophyllum hexandrum* has also been reported to modulate gamma radiation-induced immunosuppression in Balb/c mice and thus possess implications in radioprotection. Aqueous extract of *Podophyllum hexandrum* has been reported to render more than 82% survival against whole body lethal (10 Gy) gamma-irradiation in mice (Goel, Prakash et al. 2007).

The main objective of this investigation is to evaluate the protective effects of methanolic extract of *Podophyllum hexandrum* against  $\text{CCl}_4$  induced hepatic toxicity in male rats. Liver toxicity was induced by administering a single dose of  $\text{CCl}_4$  in the presence or absence of plant extract. Oxidative stress and antioxidants of liver will be studied by determination of reduced glutathione (GSH), superoxide dismutase (SOD), catalase (CAT), glutathione-S-transferase (GST), glutathione peroxidase (GPX) and glutathione reductase (GR) and lipid peroxidation (malondialdehyde).

## Materials and Methods

### Chemicals

Chemicals used in this study were of analytical grade and of highest purity procured from standard commercial sources in India. Bovine serum albumin, Folin Ciocalteu phenol reagent (E. Merk),  $\alpha$ -tocopherol, (Sigma Aldrich), Trichloroacetic acid, Sodium carbonate, Sodium dihydrogen monophosphate, Sodium hydrogen diphosphate, EDTA and Reduced nicotinamide adenine dinucleotide phosphate (NADPH) were purchased from (Hi-Media). Sodium hydroxide, reduced glutathione, 5, 5'-dithiobis-2-nitrobenzoic acid (DTNB), were obtained from (Sigma Aldrich). Methanol and Carbon tetra chloride were obtained from (E-Merk). Hydroxyl amine

hydrochloride purchased from (Qualigens).  $\alpha$ - Ketoglutarate, Aspartate, Methionine and Thiobarbituric acid (TBA) were purchased from (SRL India).

### **Plant Material Collection and Extraction**

The rhizome of *Podophyllum hexandrum* was collected from higher reaches of Aharbal in the month of May- June, and were identified by the courtesy of centre of Plant Taxonomy, Department of Botany, University of Kashmir and authenticated by a Botanist Dr. Ishad Ahmad Nawchoo (Associate professor department of Botany) and Akter Hussain Malik (Curator, centre for plant Taxonomy, University of Kashmir). The voucher specimen has been retained in the herbarium of Taxonomy department of Botany University of Kashmir for future reference under herbarium no: (KASH- bot/Ku/PH- 702- SAG).

The authentically identified plant material (rhizome) was shade dried under room temperature at  $30 \pm 2^{\circ}\text{C}$ . The dried rhizome material was grind into powder using mortar and pestle and sieved with a sieve of 0.3mm aperture size. The powder obtained was successively extracted in hexane, ethyl acetate, absolute ethanol, 70% ethanol and methanol, by using Soxhlet extractor ( $60-80^{\circ}\text{C}$ ). The methanol extract was then concentrated with the help of rotary evaporator under reduced pressure, and the solid extract was stored in refrigerator for further use.

### **Experimental animals**

Adult male albino rats of Wister strain weighing 200- 250g were purchased from the animal house of Indian Institute of Integrative and Medicine Jammu (IIIM), were used throughout this study. 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 light- and – dark cycle. The animals used in the present study were maintained in accordance with the guidelines prescribed by the National Institute of Nutrition, Indian Council of Medical Research, and the study was approved by the Ethical Committee of the Kashmir University.

**Dosage and treatment:** Rats were divided into six groups containing six rats each. The plant extract was employed at oral doses of 20, 30 and 50mg/Kg body wt. /day. The extract was suspended in normal saline such that the final volume of extract at each dose was 1ml and was fed to rats with gavage.

### **Procedure**

Group 1- Served as normal control and received olive oil only (vehicle) 5.0ml/Kg.

Group 2- Served as negative control and received  $\text{CCl}_4$  1.0ml/Kg body weight (suspended in olive oil).

Group 3-Animals were administrated with Vitamin E ( $\alpha$ -tocopherol) 50mg/Kg body weight suspended in olive oil.

Group 4- Animals received 20mg/Kg body weight of *Podophyllum* extract orally for all fifteen days.

Group 5- Animals received 30mg/Kg body weight plant extract orally for all fifteen days.

Group 6- Animals received 50mg/Kg body weight plant extract orally for all fifteen days.

On the thirteenth day, animals of the groups 2-6 were injected with CCl<sub>4</sub> at the dose of (1ml/kg body weight) intraperitoneally. After 48h of CCl<sub>4</sub> administration, the rats were sacrificed and the liver organ was isolated and PMS prepared.

The blood was collected from retro-orbital plexus without the use of anticoagulant. The blood was allowed to stand for 10 min 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).

Liver tissue was isolated from sacrificed animals were washed in ice cold 1.15% KCl and homogenized in a homogenizing buffer (50 mM Tris- HCl, 1.15% KCl pH 7.4) using Teflon homogenizer. The homogenate was centrifuged at 9,000g for 20 minutes to remove debris. The supernatant so obtained was further centrifuged at 15,000 rpm for 20 minutes at 4<sup>0</sup>C to get post mitochondrial supernatant (PMS). This PMS is used for the estimation of lipid peroxidative indices, glutathione reduced (GSH), glutathione reductase (GR), glutathione peroxidases (GPx), glutathione-S- transferase (GST), Catalase (CAT) and superoxide dismutase (SOD). Protein concentration of the supernatant was measured according to the method of Lowery et al [21] using BSA as standard.

**Serum alanine aminotransaminase (ALT):** ALT will be estimated by the method of Reitman and Frankel [22] . Briefly 0.5 ml of substrate (2mM  $\alpha$ -ketoglutarate, 0.2 M DL – alanine (in phosphate buffer 0.1 M pH 7.4 ) will be incubated at 37<sup>0</sup>C for 5 minutes. 0.1 ml of freshly prepared serum will be added to the aliquot and is again incubated at 37<sup>0</sup>C for 30 minutes. At the end of incubation 0.5 ml of 2, 4-dinitrophenylhydrazine will be added and the aliquot will be left for 30 minutes at room temperature. 0.5 ml of 0.4N NaOH will be added and the aliquot is again left for 30 minutes. Absorbance will be recorded at 505 nm against water as the blank.

**Serum aspartate aminotransaminase (AST):** AST will be estimated by the method of Reitman and Frankel [22] . Briefly 0.5 ml of substrate (2mM  $\alpha$ -ketoglutarate, 0.2 M DL – aspartate (in phosphate buffer 0.1 M pH 7.4 ) will be incubated at 37<sup>0</sup>C for 5 minutes. 0.1 ml of freshly prepared serum will be added to the aliquot and is again incubated at 37<sup>0</sup>C for 30 minutes. At the end of incubation 0.5 ml of 2, 4-dinitrophenylhydrazine will be added and the aliquot will be left for 30 minutes at room temperature. 0.5 ml of 0.4N NaOH will be added and the aliquot is again left for 30 minutes. Absorbance will be recorded at 505 nm against water as the blank.

**Serum lactate dehydrogenase (LDH):** LDH was assayed according to the method of King [23] . To 1.0 ml of buffered substrate (sodium pyruvate 37.5mM in phosphate buffer 100 mM, pH 7.4), 0.1 ml of freshly prepared serum was added and the tubes were incubated at 37<sup>0</sup>C for 15 minutes. After adding 0.2ml of NAD<sup>+</sup> solution (10mg/ml in phosphate buffer), the incubation was continued for another 15min. The reaction was arrested by adding 0.1ml of 2, 4-dinitrophenylhydrazine (0.02% in concentrated HCl), and the tubes were incubated again at 37<sup>0</sup>C for a further period of 15min, after which 7.0ml of 0.4N NaOH was added and the color developed was measured spectrophotometrically at 420nm against a blank containing phosphate buffer and DTNB only.

**Estimation of lipid peroxidation (PMS):** Lipid peroxidation in tissues was estimated by the formation of thiobarbituric acid reactive substances (TBARS) by the method of Nichans and Samuelson [24] . In brief 0.1ml of tissue homogenate (PMS; Tris- HCl buffer, pH 7.5) was treated with 2ml of (1:1:1 ratio) TBA-TCA-HCl reagent (0.37% thiobarbituric acid, 0.25 N HCl, and 15% TCA), placed in boiling water bath for 15 min, cooled and centrifuged at room temperature for 10 min. The absorbance of the clear supernatant was measured against reference blank at 535 nm.

**Determination of total sulphhydryl groups:** The acid soluble sulphhydryl groups (non protein thiols of which more than 93% is reduced glutathione (GSH) forms a yellow colored complex with DTNB that shows the absorption maximum at 412 nm. The assay procedure will be followed that of Moren et al [25]. 500µl of homogenate precipitated with 100 µl of 25% TCA, will be then subjected to centrifugation at 300xg for 10 minutes to settle the precipitate. 100 µl of the supernatant obtained shall be added to the test tube containing the 2 ml of 0.6 mM DTNB and 0.9 ml of 0.2 mM sodium phosphate buffer (pH 7.4). The yellow color obtained will be measured at 412 nm against the reagent blank which contains 100 µl of 25% TCA in place of the supernatant. Sulphydryl content shall be calculated using the DTNB molar extension coefficient of 13,100.

**Glutathione peroxidase (GPx):** GPx activity was assayed using the method of Sharma et al [26]. The assay mixture consists of 1.49 ml of sodium phosphate buffer (0.1 M p.H 7.4), 0.1 ml EDTA (1mM), 0.1 ml sodium azide (1mM), 0.1 ml 1mM GSH, 0.1ml of NADPH (0.02mM), 0.01 ml of 1mM H<sub>2</sub>O<sub>2</sub> and 0.1 ml PMS in a total volume of 2ml. Oxidation of NADPH was recorded spectrophotometrically at 340nm and the enzyme activity was calculated as nmoles NADPH oxidized/min/mg of protein, using  $\epsilon$  of  $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

**Glutathione Reductase activity (GR):** GR activity was assayed by the method of Sharma et al [26]. The assay mixture consisted of 1.6 ml of sodium phosphate buffer (0.1 M p.H 7.4), 0.1 ml EDTA (1mM), 0.1 ml 1mM oxidized glutathione, 0.1ml of NADPH (0.02mM), 0.01 ml of 1mM H<sub>2</sub>O<sub>2</sub> and 0.1 ml PMS in a total volume of 2 ml. The enzyme activity measured at 340 nm was calculated as nmoles of NADPH oxidized/min/mg of protein using  $\epsilon$  of  $6.22 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

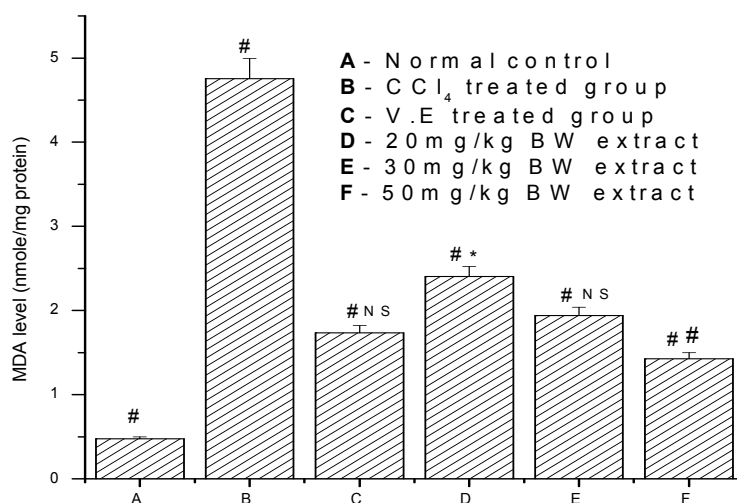
**Glutathione- S- transferase (GST) activity:** GST activity was assayed using the method of Haque et al [27]. The reaction mixture consisted of 1.67 ml sodium phosphate buffer (0.1 M pH 6.5), 0.2 ml of 1mM GSH, 0.025 ml of 1mM CDNB and 0.1 ml of PMS in a total volume of 2 ml. The change in absorbance was recorded at 340 nm and the enzyme activity was calculated as nmoles of CDNB conjugates formed/min/mg protein using  $\epsilon$  of  $9.6 \times 10^3 \text{ M}^{-1} \text{ cm}^{-1}$ .

**Catalase activity (CAT):** CAT activity was assayed by the method of Claiborne [28]. The assay mixture consisted of 1.95 ml of phosphate buffer (0.05 M pH 7), 1.0 ml H<sub>2</sub>O<sub>2</sub> (0.019 M), 0.05 ml of hepatic PMS in a final volume of 3 ml. Change in absorbance was recorded at 240 nm. CAT activity was calculated in terms of nmoles of H<sub>2</sub>O<sub>2</sub> consumed/min/mg of protein.

**Super oxide dismutase activity (SOD):** SOD activity was estimated by Beauchamp and Fridovich [29]. The reaction mixture consisted of 0.5 ml of hepatic PMS, 1 ml of 50 mM sodium carbonate, 0.4 ml of 25 µM NBT and 0.2 ml of 0.1mM EDTA. The reaction was initiated by addition of 0.4 ml of 1mM hydroxylamine- hydrochloride. The change in absorbance was recorded at 560 nm. The control was simultaneously run without tissue homogenate. Units of SOD activity were expressed as the amount of enzyme required to inhibit the reduction of NBT by 50%.

## Results

**Effect of tested extract on liver peroxidation:** The effect of tested extract on CCl<sub>4</sub> induced LPO was examined through monitoring the levels of MDA. After CCl<sub>4</sub> administration, the MDA level increased significantly from  $0.476 \pm 0.148$  to  $4.755 \pm 0.425$  nm/mg protein. However, pretreatment with the methanolic extract of *Podophyllum hexandrum* at a concentration of 20, 30 and 50mg/kg BW, the MDA level decreased to 2.403, 1.940 and 1.426 from the control, with the percentage inhibition of 49.51, 59.261 and 70.083% respectively (Fig. 1).



**Fig. 1:** #;  $p < 0.001$  compared with CCl<sub>4</sub> and normal control, \*;  $p < 0.05$  and NS once compared with normal control only. The data were presented as means  $\pm$  S.D of six parallel measures and evaluated by one way ANOVA followed by the Dunnett's – test to detect inter group differences. Differences were considered to be statistically significant if  $p < 0.05$ .

#### Effect of tested extract on liver marker enzymes:

Table 1 depicts the significant ( $p < 0.001$ ) elevation of liver diagnostic marker enzymes, ALT ( $124.6013 \pm 14.90$  U/L), AST ( $134.248 \pm 11.358$  U/L) and LDH ( $242.77 \pm 14.953$  U/L) in the serum of rats after CCl<sub>4</sub> administration (group II) as compared to normal rats (group I). Oral pretreatment with methanolic extracts of *Podophyllum hexandrum* at a higher concentration of 50mg/kg body weight, for a period of 15 days resulted in retaining the level to ( $72.083 \pm 8.590$  U/L), ( $70.742 \pm 9.642$  U/L) and ( $185.852 \pm 8.776$  U/L) respectively.

#### Reduced Glutathione levels (GSH):

Table 2 shows the cellular GSH data of liver tissue homogenate. A significant ( $P < 0.001$ ) decrease in the GSH was observed in the group II animals treated with CCl<sub>4</sub> ( $17.473 \pm 3.15$ nmoles/g protein respectively), as compared to the respective normal control group ( $112.59 \pm 1.56$ nmoles/g protein). Administration of methanolic extract of *Podophyllum hexandrum* for 15 consecutive days afforded a dose dependent protection against such depletion of GSH level. Vitamin E a known antioxidant restores the GSH content to a still larger extent.

#### Glutathione reductase and Glutathione Peroxidase levels

Significant decrease was observed in CCl<sub>4</sub> administered rats when compared with that of the normal control animals. Treatment with methanolic extract of *Podophyllum hexandrum* at the oral dose of 20, 30 and 50mg/kg body weight significantly restored the level of both enzymes in the liver tested organ (Table 2).

**Table 1: Effect of Podophyllum hexandrum methanolic extract on ALT, AST and LDH levels in CCl<sub>4</sub> induced hepatotoxicity in albino rats.**

Treatment	Dose mg/kg	ALT U/L	AST U/L	LDH U/L
Control group (Olive oil only)	5 ml/Kg	27.0662 ± 6.852 <sup>#</sup>	21.970 ± 5.047 <sup>#</sup>	46.351 ± 8.032 <sup>#</sup>
CCl <sub>4</sub> treated group	1 ml/Kg	124.6013 ± 14.90 <sup>#</sup>	134.248 ± 11.358 <sup>#</sup>	242.77 ± 14.953 <sup>#</sup>
CCl <sub>4</sub> treated + V.E	50 mg/Kg	55.567 ± 5.887 <sup>#</sup>	58.678 ± 5.816 <sup>#a</sup>	170.421 ± 5.336 <sup>#NS</sup>
CCl <sub>4</sub> treated + <i>P.H</i> extract	20 mg/Kg	85.209 ± 4.192 <sup>#</sup>	100.00 ± 3.838 <sup>#*</sup>	209.973 ± 4.342 <sup>#**</sup>
CCl <sub>4</sub> treated + <i>P. H</i> extract	30 mg/kg	71.275 ± 5.900 <sup>#</sup>	75.026 ± 6.793 <sup>#a</sup>	189.068 ± 3.702 <sup>#NS</sup>
CCl <sub>4</sub> treated + <i>P. H</i> extract	50 mg/kg	72.083 ± 8.590 <sup>**</sup>	70.742 ± 9.642 <sup>#*</sup>	185.852 ± 8.776 <sup>#NS</sup>

<sup>#</sup>; p < 0 .001 compared with CCl<sub>4</sub>, a; p < 0 .001 compared with normal control, \*; p < 0 .05, \*\*; P < 0.01 and NS once compared with normal and CCl<sub>4</sub> group. The data were presented as means ± S.D of six parallel measures and evaluated by one way ANOVA followed by the Dunnett's – test to detect inter group differences. Differences were considered to be statistically significant if p < 0 .05.

### Superoxide dismutase levels (SOD)

The activities of SOD in the liver tissue homogenate are shown in Table 2. In liver homogenate CCl<sub>4</sub> treatment caused reduction of the SOD activity to a level of 21.970 ± 2.679 from 46.850 ± 7.795. Methanolic extract restores the level to 30.898 ± 14.006, 43.305 ± 3.266 and 44.950 ± 6.100 at a dosage of 20, 30 and 50mg/kg BW.

### Catalase levels

CAT activities in the liver homogenate of rats for all experimental groups are shown in Table 2. The CAT activity observed in the liver tissue of CCl<sub>4</sub> treated rats was considerably lower (153.66 ± 9.07 nmoles/min/mg protein) as compared to normal group (2488.57 ± 113.16 nmoles/min/mg protein). In the pretreatment groups, receiving methanolic extract of *Podophyllum hexandrum* for 15 days prior to CCl<sub>4</sub>, CAT activity was significantly increased in a dose dependent manner. At a concentration of 50 mg/kg body weight the level was restored back to 1490.73 ± 51.15 nmoles/mg protein in liver tissue.

### Glutathione- S- transferase levels

The effect of oxidant and antioxidants on Glutathione- S- transferase (GST) activity in liver tissue homogenate of all the experimental animals have been shown in Table 2. The GST activity was decreased in CCl<sub>4</sub> treated groups compared to the normal group. Only about 7.030 ± 0.231 nmoles/min/mg protein of GST were observed in CCl<sub>4</sub> treated group, as compared to the value of 11.364 in the normal controls. Pretreatment of methanolic extract largely restored the GST activity in a dose dependent manner.

**Table 2: Shows the effect of methanolic extract of *Podophyllum 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 extract	Group V 30 mg/kg extract	Group VI 50 mg/kg extract
Reduced sulphhydryl group	112.59 ± 1.56 <sup>a</sup>	17.473 ± 3.15 <sup>b</sup>	107.24 ± 2.42 <sup>ac</sup>	91.273 ± 3.13 <sup>ab</sup>	97.021 ± 2.85 <sup>ab</sup>	105.086 ± 2.74 <sup>ab</sup>
Glutathione reductase	31.236 ± 1.528 <sup>a</sup>	2.208 ± 0.095 <sup>b</sup>	28.574 ± 1.565 <sup>ac</sup>	9.503 ± 0.778 <sup>ab</sup>	15.987 ± 0.827 <sup>ab</sup>	18.564 ± 1.713 <sup>ab</sup>
Glutathione peroxidase	36.810 ± 5.134 <sup>a</sup>	2.208 ± 0.095 <sup>b</sup>	30.906 ± 1.552 <sup>ab</sup>	10.817 ± 0.279 <sup>ab</sup>	19.219 ± 0.0814 <sup>ab</sup>	22.653 ± 1.087 <sup>ab</sup>
Superoxide dismutase	46.850 ± 7.795 <sup>a</sup>	21.970 ± 2.679 <sup>b</sup>	45.095 ± 10.44 <sup>ac</sup>	30.898 ± 14.006 <sup>f*</sup>	43.305 ± 3.266 <sup>ac</sup>	44.950 ± 6.100 <sup>ac</sup>
Catalase activity	2488.57 ± 113.16 <sup>a</sup>	153.66 ± 9.07 <sup>b</sup>	2098.95 ± 98.41 <sup>ab</sup>	734.81 ± 26.54 <sup>a,b</sup>	1227.77 ± 39.12 <sup>a,b</sup>	1490.73 ± 51.15 <sup>ab</sup>
Glutathione- S-transferase	11.364 ± 0.452 <sup>a</sup>	7.030 ± 0.231 <sup>b</sup>	10.704 ± 0.140 <sup>ac</sup>	7.160 ± 0.682 <sup>ab</sup>	9.242 ± 0.589 <sup>ab</sup>	10.636 ± 0.544 <sup>ac</sup>

**a:**  $p < 0.001$  as compared with CCl<sub>4</sub> induced group, **b:**  $p < 0.001$  as compared with normal group, **c:**  $p < 0.01$  as compared with normal group, **d:** NS as compared with CCl<sub>4</sub> induced group, **e:** NS as compared with normal group, **f:**  $p < 0.01$  as compared with CCl<sub>4</sub> induced group and **\***:  $p < 0.05$  as compared with normal group.

The data were presented as means ± SD for six animals in each observation and evaluated by one-way ANOVA followed by Dunnett's test to detect inter group differences. Differences were considered to be statistically significant if  $p < 0.05$ .

### Discussion

Ample experimental and epidemiological studies support the involvement of oxidative stress in the pathogenesis and progression of several chronic diseases [30]. It is now known that oxygen, indispensable for maintaining life, sometimes becomes toxic and results in the generation of most aggressive agents such as reactive oxygen species (ROS). CCl<sub>4</sub> – mediated hepatotoxicity was taken here as the experimental model for liver injury. It has been established that CCl<sub>4</sub> is accumulated in hepatic parenchymal cells and metabolically activated by cytochrome P-450 dependent monooxygenases to form a trichloromethyl free radical (CCl<sub>3</sub>·) which alkylates cellular proteins and other macromolecules with a simultaneous attack on polyunsaturated fatty acids in the presence of oxygen to produce lipid peroxides leading to liver damage [31]. The aim of this investigation was to study the protective effects of rhizome methanolic extract of *Podophyllum hexandrum* on CCl<sub>4</sub> induced liver toxicity. The CCl<sub>4</sub> induced liver lesion was associated with massive elevation in liver MDA level. MDA elevation is a result of oxidative stress demonstrated here through the decrease of total antioxidant capacity, GSH level, and antioxidant enzymes. MDA produced as by product of LPO that occurs in hydrophobic core of biomembranes [32]. The significant decline in the concentration of these constituents in the liver tissue of CCl<sub>4</sub> + methanolic extract administered rats indicates antilipid peroxidative effect of *Podophyllum hexandrum*.

It is a natural phenomenon to observe that as and when liver cell plasma membrane gets damaged by the free radicals, a variety of marker enzymes, like aspartate aminotransferase (AST), alanine aminotransferase (ALT) and lactate dehydrogenase (LDH) normally located in



the cytosol would be released into the blood stream. Their estimation in the serum thus could be a useful quantitative marker of the extent and type of hepatocellular damage [33]. Methanolic extract of *Podophyllum hexandrum* in a dose dependent manner decreased the levels of AST, ALT and LDH significantly in CCl<sub>4</sub> treated rats indicating maintenance of functional integrity of hepatic cell membrane.

The body has an effective mechanism to prevent and neutralize the free radical induced damage. This is accomplished by a set of endogenous antioxidant enzymes, such as SOD, CAT, GPX and GR etc. When the balance between ROS production and antioxidant defenses is lost, 'oxidative stress' results, which through a series of events deregulates the cellular functions leading to various pathological conditions [34]. Any compound, natural or synthetic, with antioxidant properties might contribute towards the partial or total alleviation of this type of damage. GSH is a major non-protein thiol in living organisms which plays a central role in coordinating the body's antioxidant defense processes. Perturbation of GSH status of a biological system has been reported to lead to serious consequences [34]. Decline in GSH content in the liver of CCl<sub>4</sub> intoxicated rats, and its subsequent return towards near normalcy in CCl<sub>4</sub> + methanol treated rats reveal antioxidant effect of *Podophyllum hexandrum*. SOD, CAT and GPX constitute a mutually supportive team of defense against ROS. SOD is a metalloprotein and is the first enzyme involved in the antioxidant defense by lowering the steady-state level of O<sub>2</sub><sup>-</sup>. CAT is a heme protein, localized in the peroxisomes or the microperoxisomes. This enzyme catalyses the decomposition of H<sub>2</sub>O<sub>2</sub> to water and oxygen and thus protecting the cell from oxidative damage by H<sub>2</sub>O<sub>2</sub> and OH<sup>•</sup>. GPX is a seleno-enzyme two third of which (in liver) is present in the cytosol and one third in the mitochondria. It catalyses the reaction of hydroperoxides with reduced glutathione to form glutathione disulphide (GSSG) and the reduction product of the hydroperoxide. In our study, decline in the activities of these enzymes in CCl<sub>4</sub> administered rats revealed that LPO and oxidative stress elicited by CCl<sub>4</sub> intoxication have been nullified due to the effect of methanolic extract of *Podophyllum hexandrum*. GTS plays an essential role in liver by eliminating toxic compounds by conjugating them with glutathione. GR is concerned with the maintenance of cellular level of GSH (especially in the reduced state) by effecting fast reduction of oxidized glutathione to reduced form. The activities of both these enzymes were found to be restored an almost normal activity in CCl<sub>4</sub> + methanolic administered rats, thus unearthing the antioxidant effect of *Podophyllum hexandrum*. Natural antioxidants strengthen the endogenous antioxidant defenses from ROS ravage and restore the optimal balance by neutralizing the reactive species. They are gaining immense importance by virtue of their critical role in disease prevention.

In conclusion, the methanolic extract of rhizomes of *Podophyllum hexandrum* exhibit a liver protective effect against CCl<sub>4</sub> induced hepatotoxicity and shows anti-lipid peroxidative and antioxidant activities.

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