

## Nephroprotective potential of *Pistacia chinensis* bark extract against induced toxicity in rats

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**Abstract.** Sattar S, Khan MR, Shah NA, Noreen F, Naz K. 2016. Nephroprotective potential of *Pistacia chinensis* bark extract against induced toxicity in rats. *Nusantara Bioscience* 8: 192-200. *Pistacia chinensis* possesses profound antioxidant properties. In this study, the protective influence of *P. chinensis* bark ethanol extract (PCBE) was scrutinized against CCl<sub>4</sub> induced renal toxicity in rats (6 rats in each group). Seven different groups of Sprague–Dawley male rats were intraperitoneally injected with CCl<sub>4</sub> (1 mL/kg b.w.; 30% CCl<sub>4</sub> in olive oil) at an interval of 48 hour for four weeks. PCBE at doses of 200 and 400 mg/kg b.w. or silymarin at a dose of 100 mg/kg b.w. was orally administered to the animals on alternate days. CCl<sub>4</sub> induced renal toxicity was evident by a significant increase in specific gravity, albumin, count of RBCs and pus cells in urine. Administration of PCBE significantly ameliorated the enhanced serum level of urea, creatinine, and bilirubin whereas increased the level of total protein. Moreover, the influence of CCl<sub>4</sub> significantly elevated the level of lipid peroxidation (TBARS), H<sub>2</sub>O<sub>2</sub> and nitrite content whereas enzymatic antioxidants (glutathione-S-transferase, glutathione peroxidase, glutathione reductase, catalase, superoxide dismutase, peroxidase,  $\gamma$ -glutamyl transpeptidase, and quinone reductase), non-enzymatic antioxidant reduced glutathione (GSH) content in kidney homogenate was compromised. When animals were treated along PCBE, a remarkable protective role was observed on all the parameters of kidney in comparison to only CCl<sub>4</sub> treated groups. From this study, it can be concluded that PCBE holds an effective antioxidant and nephroprotective property.

**Keywords:** Lipid peroxidation, renal damage, glutathione, antioxidant, creatinine

### INTRODUCTION

Medicinal plants are considered an important source of therapeutic agents, both in folk and modern medicines (Krentz and Bailey 2005). According to an estimation of the World Health Organization (WHO 2002) about 75-80% population is dependent on the use of medicines derived from plants as a remedy of their ailments. Another study marked out that almost 25-30% prescribed medicines and about 11% of crucially important drugs are plants derived. Moreover, enormous other medicines are also synthesized from precursor composites obtained from plants (Rates 2001). The indigenous people of developing countries immensely rely on traditional medicines. The utilization of plants as therapeutic agents is an old practice and most of the drugs available in market are directly or indirectly plant-derived (Yuan et al. 2010). The weak socioeconomic position and inadequate medical facilities available to natives of rural areas compel them to rely on conventional drugs (Corrêa et al. 2011).

Fibrosis is a clinically challenging and frequently occurring process due to chronic tissue injury (Benyon and Iredale 2000). The potent agent usually used to prompt fibrosis in animal models is CCl<sub>4</sub> (Armendariz-Borunda et al. 1990). The major cause of chronic kidney disorder is renal fibrosis (Pradère et al. 2008). Such renal fibrosis also results from acute diabetes, ureteral impediment and hypertension (Al-Bayati et al. 2002). Changes in renal

vascular structure and glomerulosclerosis are also the cause of renal fibrosis (Pradère et al. 2008).

The loss of balance between the Reactive Oxygen Species (ROS) and the defensive machinery of a cell leads to the creation of oxidative stress resulting in worse damage to the cell (Samouilidou et al. 2003). In a cell or tissue various Nitrogen Reactive Species (RNS) and ROS are evolved, either as the result of normal cell accomplishments or exogenous influences such as chemicals, UV radiations, ionizing beams, and sunlight. Here, OH free ions, H<sub>2</sub>O<sub>2</sub>, and superoxides are eminent reactive oxygen species (Cerutti 1991).

To induce oxidative stress in experimental organisms normally CCl<sub>4</sub> is used. Free radicals are produced in various tissues such as kidneys, blood, brain, liver, heart, lungs and glands by CCl<sub>4</sub> (Pirinçioğlu et al. 2012). Tissue injury tempted by CCl<sub>4</sub> is channeled through the development of highly reactive intermediates; •CCl<sub>3</sub> (trichloromethyl radical) and CCl<sub>3</sub>OO• (trichloromethyl peroxy radical). These radicals are produced by cytochrome (P-450) of tissue and are assumed to cause peroxidation of lipids (Shah et al. 2013). Further on, ROO• (peroxy radicals) and R• (alkoxy) radicals are generated by the reaction between PUFA (polyunsaturated fatty acids) and free radicals such as •CCl<sub>3</sub> and CCl<sub>3</sub>OO•. These alkoxy and peroxy radicals latter on produces lipid peroxides which are considered to be extremely reactive, altering

enzymatic functions of a cell and leads to induction of necrosis (Khan et al. 2010).

Various abnormalities in cells, such as imbalance of cellular metabolism, alterations in nucleic acid and protein structures, shift in cellular levels of free calcium, damage to cell membrane permeability and cellular mutilation by lipid peroxidation are induced with oxidative stress. Currently, lipid peroxidation is vastly gaining attention because of its high association with various abnormal physiological reactions (Cabre et al. 2000). Protection against such free oxidative stress causing radicals is itself present in nature in form of different plants containing a number of various medicinally important constituents, inhibited the activity of certain hazardous enzymes activated by  $\text{CCl}_4$  (Karakus et al. 2011). Many bioactive compounds of medicinal plants possess free radicals scavenging activity and are beneficial in removal of such stress producing toxins (Sahreen et al. 2010). Ascorbic acid, monosaccharides, and polyphenolics are endogenous antioxidants and are involved in establishment of cellular stability by removal of free radicals. These natural antioxidants work as a shield against the attacks of free radicals that can be the cause of various irreversible damages to the cell. Supplementation of more than one antioxidant can reduce the expanse of free radical damage. Floral resources possess quite a large number of various antioxidant compounds involved in the scavenging of free radicals (Khan et al. 2009).

*Pistacia chinensis* is of much important from medicinal point of view as in Pakistan and widely used as an anti-depressant, anti-inflaming agent and an analgesic (Ahmad et al. 2010). As a folk medicine, it is found to have therapeutic effect for the cure of hepatitis and liver diseases. However, the *in vivo* activity of *P. chinensis* as antioxidant is not attempted yet. This study was processed to evaluate antioxidative effects of *P. chinensis* trunk bark ethanolic extract against  $\text{CCl}_4$  induced renal toxicity.

## MATERIALS AND METHODS

### Chemicals

Reduced glutathione (GSH), glutathione reductase,  $\gamma$ -glutamyl p-nitro anilide, bovine serum albumin (BSA), 1,2-dithio-bis nitro benzoic acid (DTNB), 1-chloro-2,4-dinitrobenzene (CDNB), reduced nicotinamide adenine dinucleotide phosphate (NADPH), flavin adenine dinucleotide (FAD), glucose-6-phosphate, 2,6-dichlorophenolindophenol, thiobarbituric acid (TBA), picric acid, sodium tungstate, sodium hydroxide, trichloroacetic acid (TCA),  $\text{CCl}_4$  were purchased from Sigma Chemicals Co. USA.

### Plant collection and extraction

The trunk bark of *P. chinensis* was collected in November 2012 from the campus of Quaid-i-Azam University Islamabad Pakistan. It was authenticated by Dr. Zafer, Curator of National Herbarium of Quaid-i-Azam University, Pakistan and a herbarium voucher no. 45675 was allocated. The bark was washed properly and dried in

the shade for three weeks. Electric grinder was utilized to crush the dried bark of plant to powder form. Extraction in ethanol was carried out (twice) by mixing 2 kg of bark powder in 4 liters of crude ethanol at 25°C for 48 h. To filter the extract filter paper (Whatman No. 1) was used. In the next step, filtrate was concentrated through rotary evaporator at reduced pressure of 40°C. The dried extract was refrigerated at 4°C for further use for carrying out *in vivo* assays.

### Experimental design

Sprague Dawley rats (6 rats in each group) weighing about 150-200 g were placed in regular rat cages at room temperature 25-30°C with normal 12 h light and dark cycles. The experimental proposal was ratified by a committee of ethical issues, Quaid-i-Azam University Islamabad.

Prior preceding the experiment animals were allowed to habituate the environment and were fed with standard laboratory food i.e. 35% protein; 21.05%, ash; 8.3%, carbohydrates; 52.06%, fiber; 3.07, phosphorous; 0.55, sand (silica); 1.65%, moisture; 10% (w/w), calcium; 0.9% , and freshwater with *ad libitum*. Forty-two rats were equally divided into seven groups for the experiment after weighing them to confirm their health conditions. The laboratory chemical carbon tetrachloride (30%) was suspended in edible olive oil (70%). The dose administrated to induce toxicity was calculated according to the body weight of the experimental rat. A calculated quantity of ethanol extract of *P. chinensis* bark was dissolved in DMSO and the amount of extract given intraperitoneally was dependent on the body weight of the experimental rat. To induce  $\text{CCl}_4$  toxicity (Shyu et al. 2008) protocol was followed with some changes. Carbon tetrachloride (1 mL/kg b.w.) was injected in the intraperitoneum of rats on alternative days for 4 weeks. Besides, silymarin (100 mg/kg b.w.), low dose (200 mg/kg b.w.) and high dose (400 mg/kg b.w.) of *P. chinensis* ethanol extract of bark extract were given orally along with  $\text{CCl}_4$  according to the following pattern.

Group I: Treated as control, received no dose.

Group II: Olive oil (1 mL/kg b.w.) + DMSO (1 mL/kg b.w.)

Group III:  $\text{CCl}_4$  (1 mL/kg b.w.).

Group IV:  $\text{CCl}_4$  (1 mL/kg b.w.) + silymarin (100 mg/kg b.w.)

Group V:  $\text{CCl}_4$  (1 mL/kg b.w.) + PCBE (200 mg/kg b.w.)

Group VI:  $\text{CCl}_4$  (1 mL/kg b.w.) + PCBE (400 mg/kg b.w.)

Group VII: PCBE (400 mg/kg b.w.)

Four weeks later, animals were shifted in metabolic cages and after 24 h of last treatment, urine was collected in Eppendorf and stored at -70°C for urine analysis. All the animals were sacrificed at the end of the experiment by giving chloroform anesthesia according to the weight of animal and dissected from ventral side. Blood was drawn by the cardiac puncture using 23G1 syringe in a falcon tube for serum tests after performing centrifugation at 10,000 rpm for 15 min at 4°C. The kidneys were removed and washed with ice-cold saline to remove debris and stored in liquid nitrogen at -70°C for tissue homogenate tests. Small parts of organs were fixed with 10% phosphate-buffered formalin for histology.

### Histopathological study of renal tissue

Fixed tissues were embedded in parafilm and 3-4  $\mu\text{m}$  thin sections were stained with hematoxylin-eosin and photographed under light microscope (DIALUX 20 EB) at 40X.

### Urine analysis

By using standard diagnostic kits (Krenngasse 12, 8010 Graz, Australia), urine samples were analyzed for estimation of leukocytes, red blood cells count (RBCs), albumin, pH and specific gravity.

### Serum analysis

Creatinine, bilirubin, and urea of serum samples were estimated with the help of AMP diagnostics company kits (Krenngasse 12, 8010 Graz, Australia).

### Estimation of antioxidant enzymes

Kidney tissues from each sample of various treatments were homogenized in 1 ml of potassium phosphate buffer (100 mM) mixed with EDTA (1 mM) and maintained at pH 7.4. The homogenate was further centrifuged at  $12000 \times g$  at  $4^\circ\text{C}$  for 30 min to obtain the supernatant for following antioxidant enzyme assays.

#### Catalase (CAT) activity

Activity of CAT was determined by following the method of Kakkar, Das et al. (1984). The evaluation of CAT activity relies on the mechanism of  $\text{H}_2\text{O}_2$  splitting into free radicals. The absorbance of 25  $\mu\text{l}$  of tissue homogenate supernatant was measured at 240 nm after one min of incubation with 625  $\mu\text{l}$  of potassium phosphate buffer (50 mM, pH 5.0) and 100  $\mu\text{l}$  of  $\text{H}_2\text{O}_2$  (5.9 mM). The one unit catalase activity is described as the change in absorbance of 0.01 as units/min.

#### Peroxidase (POD) activity

Peroxidase activity was measured spectrophotometrically by the method of Kakkar, Das et al. (1984) with slight modification. An aliquot of 25  $\mu\text{l}$  of tissue supernatant was added to 75  $\mu\text{l}$  of 40 mM  $\text{H}_2\text{O}_2$ , 25  $\mu\text{l}$  of 20 mM guaiacol and 625  $\mu\text{l}$  of 50 mM potassium phosphate buffer (pH 5.0). After one min change in absorbance was measured at 470 nm. Change in absorbance of 0.01 as units/min, is defined as one unit POD activity.

#### Superoxide dismutase (SOD) activity

Protocol of Kakkar, Das et al. (1984) was used to find out the SOD activity. Measurement of SOD activity was carried out by using sodium pyrophosphate buffer and phenazine methosulphate. Tissue homogenate was centrifuged for 10 min at  $1500 \times g$  followed by  $10000 \times g$  for 15 min. Supernatant was collected and used to determine SOD activity. An aliquot containing 150  $\mu\text{l}$  of supernatant was added to 600  $\mu\text{l}$  of 0.052 mM sodium pyrophosphate buffer (pH 7.0) and 50  $\mu\text{l}$  of 186  $\mu\text{M}$  phenazine methosulphate. To the mixture 100  $\mu\text{l}$  of 780  $\mu\text{M}$  NADH was added to start enzymatic reaction. Addition of 500  $\mu\text{l}$  of glacial acetic acid after 1 min stops the reaction.

Optical density was determined at 560 nm to measure the color intensity. Results were expressed in units/mg protein.

#### Glutathione peroxidase (GSH-Px) activity

Sahreen, Khan et al. (2013) protocol was followed to investigate the glutathione peroxidase activity. GSH-Px assay was carried out by using NADPH as substrate. An aliquot of 50  $\mu\text{l}$  of tissue supernatant was added to 740  $\mu\text{l}$  of 0.1 M sodium phosphate buffer (pH 7.4), 50  $\mu\text{l}$  of 1 mM sodium azide, 25  $\mu\text{l}$  of glutathione reductase (1 unit/ml), 50  $\mu\text{l}$  of 1 mM EDTA, 5  $\mu\text{l}$  of 0.25 mM  $\text{H}_2\text{O}_2$ , 50  $\mu\text{l}$  of 0.2 mM NADPH and 25  $\mu\text{l}$  of 1 mM GSH mixture. Blank reaction tube included distilled water instead of tissue supernatant. With the help of spectrophotometer oxidation of NADPH was measured at 340 nm. Using molar coefficient of  $6.23 \times 10^3/\text{M}/\text{cm}$ , GSH-Px activity was determined as amount of NADPH oxidized/min/mg protein.

#### Glutathione Reductase (GSR) activity

Sahreen, Khan et al. (2013) procedure was followed to find out the glutathione reductase activity. GSR activity was determined by using NADPH as substrate. An aliquot of 50  $\mu\text{l}$  of tissue supernatant was added to 50  $\mu\text{l}$  of 0.5 mM EDTA, 50  $\mu\text{l}$  of 0.1 mM NADPH, 25  $\mu\text{l}$  of 1 mM oxidized glutathione and 825  $\mu\text{l}$  of 0.1 M sodium phosphate buffer (pH 7.6) mixture. With the help of spectrophotometer, decomposition of NADPH is measured at 340 nm at  $25^\circ\text{C}$ . Using molar extinction coefficient of  $6.23 \times 10^3/\text{M}/\text{cm}$ , GSR activity was determined as amount of NADPH oxidized/min/mg protein.

#### Glutathione-S-transferase (GST) activity

Sahreen et al. (2013) protocol was used to estimate glutathione-S-transferase activity. GST assay was based on the formation of CDNB conjugate. An aliquot of 150  $\mu\text{l}$  of tissue supernatant was added to a mixture of 100  $\mu\text{l}$  of 1 mM reduced glutathione, 12.5  $\mu\text{l}$  of 1 mM CDNB and 720  $\mu\text{l}$  of sodium phosphate buffer. Optical density was measured at 340 nm. Using a molar coefficient of  $9.61 \times 10^3/\text{M}/\text{cm}$ , GST activity was measured as amount of CDNB conjugate formed/min/mg protein.

#### Quinone reductase (QR) activity

Method of Shah and Khan (2014) was followed to determine quinone reductase activity. Quinone reductase activity is measured by a method which is based on reduction of dichlorophenol indophenol complex. An aliquot of 33.3  $\mu\text{l}$  of tissue homogenate was added to 233  $\mu\text{l}$  of bovine serum albumin, 6.6  $\mu\text{l}$  of 0.1 mM NADPH, 33.3  $\mu\text{l}$  of 50 mM FAD and 710  $\mu\text{l}$  of 25 mM Tris-HCl buffer (pH 7.4). Optical density was measured at 600 nm. Using molar extinction coefficient of  $2.11 \times 10^4/\text{M}/\text{cm}$ , QR activity was determined as nM of DCPIP reduced/min/mg protein.

#### $\gamma$ -Glutamyl transpeptidase ( $\gamma$ -GT) activity

Shah and Khan (2014) method was followed to find out the  $\gamma$ -glutamyl transpeptidase activity. To determine the activity of  $\gamma$ -GT, glutamyl nitroanilide was used as a

substrate. An aliquot of 50  $\mu$ l of tissue homogenate was added to mixture containing 250  $\mu$ l of 4 mM glutamyl nitroanilide, 250  $\mu$ l of 40 mM glycylglycine and 250  $\mu$ l of 11 mM  $MgCl_2$  (prepared in 185 mM Tris HCl buffer) at room temperature. Addition of 250  $\mu$ l of 25% trichloroacetic acid after 10 min of incubation stopped the reaction. Resulting mixture was centrifuged at  $2500 \times g$  for 10 min. Supernatant was collected and optical density was measured at 405 nm. Using a molar extinction coefficient of  $1.75 \times 10^3/M/cm$ ,  $\gamma$ -GT activity was determined as nM nitroaniline formed/min/mg protein.

### Estimation of biochemical parameters

#### Protein estimation

The protocol of Khan et al. (2015) was used to estimate the total soluble proteins present in the tissue. To 0.1 ml of each supernatant sample, 1 ml of alkaline solution was added afterward, it was mixed thoroughly by vortex machine. The optical density was recorded at 595 nm after an incubation of 30 min. Bovine serum albumin curve was used to Figure out the concentration of total serum proteins present in the sample.

#### Estimation of lipid peroxidation assay (TBARS)

The assay for lipid peroxidation was carried out by following the modified method of Khan, Khan et al. (2015). The reaction mixture comprised of 0.58 ml of 0.1 M phosphate buffer (pH 7.4), 0.2 ml of 100 mM ascorbic acid, 0.02 ml of 100 mM ferric chloride and 0.2 ml of sample homogenate and was incubated at 37°C for 60 min in shaking water bath. 1.0 ml of 10% trichloroacetic was added to stop the reaction, followed by addition of 1.0 ml thiobarbituric acid (0.67%). Firstly, the tubes containing reaction mixture were placed in water bath at 100°C for 15-20 min and then they were transferred to crushed ice bath. Later, they were centrifuged at  $2500 \times g$  for 10-15 min. Optical density was determined at 535 nm against a blank containing reagent. The results were accounted as nM TBARS/min/mg tissue at 37°C via molar extinction coefficient of  $1.56 \times 10^5/M/cm$ .

#### Estimation of reduced glutathione (GSH) content

Concentration of GSH was measured according to method of Naz, Khan et al. (2014). An aliquot of 500  $\mu$ l of tissue supernatant was precipitated by addition of 500  $\mu$ l of sulfosalicylic acid (4%). After 1 h of incubation at 4°C, samples were centrifuged for 20 min at  $1200 \times g$ . A volume of 33  $\mu$ l of supernatant was collected and added to aliquots containing 900  $\mu$ l of 0.1 M potassium phosphate buffer (pH 7.4) and 66  $\mu$ l of 100 mM DTNB. Reduced glutathione reacts with DTNB and forms a yellow colored complex. Absorption was measured at 412 nm and GSH contents were estimated as  $\mu$ M GSH/g tissue.

#### Estimation of hydrogen peroxide ( $H_2O_2$ )

Naz et al. (2014) protocol was followed to perform hydrogen peroxide ( $H_2O_2$ ) assay. The oxidation of phenol red was carried out by  $H_2O_2$ -mediated horseradish peroxidase enzyme. To 1 ml of phenol red (0.28 nM) solution, 2.0 ml of sample supernatant, 5.5 nM dextrose,

0.05 M phosphate buffer (pH 7), horseradish peroxidase (8.5 units) was added and incubated for 60 min at 37°C. To stop the reaction 0.01 ml of 10 N NaOH was added and centrifugation was done at  $800 \times g$  for 5-10 minutes. The absorbance of sample was noted at 610 nm by using the reagent as a blank. The concentration of  $H_2O_2$  was given as nM  $H_2O_2$ /min/mg tissue based on the standard curve of  $H_2O_2$  oxidized phenol red.

#### Estimation of nitrite content

Griess reagent was used to accomplish the nitrite assay (Shah et al. 2013). Equal quantities of 5%  $ZnSO_4$  and 0.3 M NaOH were utilized to deproteinize the supernatant and then centrifugation was done at  $6400 \times g$  for 15-20 min to gain the supernatant. To blank the spectrophotometer 1 ml of Griess reagent was used and the absorbance of samples was evaluated at 540 nm by adding 20  $\mu$ l of supernatant to a cuvette having Griess reagent. Standard curve of sodium nitrite was used to find out the concentration of nitrite.

### Statistical analysis

The values were expressed as means  $\pm$  standard deviation (SD) of six observations in each group. Graph PadPrism5 and Biostatistix software were used to calculate different parameters of the study. To analyze significance level between different treated groups post hoc comparison was estimated at  $P=0.05$ .

## RESULTS AND DISCUSSION

Chronic nephrotoxicity was induced with carbon tetrachloride. This study was conducted to evaluate the pharmacological activity of plant extracts against  $CCl_4$  triggered oxidative stress. In this context, various parameters of renal system were analyzed, i.e., urine profile, serum profile, antioxidant enzymatic, non-antioxidant enzymatic levels and histomorphological changes.

The main objective of carrying out this study was to assess the pharmacological potential of *P. chinensis* bark ethanol extract against  $CCl_4$  induced nephrotoxicity in rats.

### Effect of PCBE on urine profile of rats

The protective effects of PCBE against carbon tetrachloride-induced oxidative stress on urine profile, i.e. pH, specific gravity, leukocytes, RBC, albumin was minutely studied. The induction of  $CCl_4$  resulted in the decrease ( $P<0.05$ ) of pH, whereas specific gravity, count of pus cells, red blood cells, albumin were significantly increased ( $P<0.05$ ) in urine of rats (Table 1). PCBE remarkably diminished ( $P<0.05$ ) the toxic effects of  $CCl_4$  by achieving the values of all above parameter near to that of the control. The olive oil + DMSO and silymarin +  $CCl_4$  showed the values of all the parameters almost equal ( $P<0.05$ ) to control values. The high and low dose of PCBE were observed to keep the concentrations equal ( $P<0.05$ ) to that of the control. However, the dose containing only PCBE acted as normal.

### Effect of PCBE on serum profile of rats

The effect of PCBE on serum markers test (urea, direct bilirubin, and creatinine) against CCl<sub>4</sub> induced toxicity in rats was also observed. The level of urea, direct bilirubin and creatinine were considerably increased ( $P < 0.05$ ) in case of 1 mL/kg b.w of CCl<sub>4</sub> treated rats (Table 2). Administration of PCBE dose-dependently ameliorated ( $P < 0.05$ ) the effects of CCl<sub>4</sub> near to the control. When treated with low dose (200 mg/kg b.w.) of PCBE the urea, creatinine and bilirubin levels were sustained as compared to the CCl<sub>4</sub> treated animals (urea =  $72.9 \pm 6.8$  mg/dl, creatinine =  $1.42 \pm 0.25$  mg/dl and bilirubin =  $1.97 \pm 0.26$  mg/dl). The high dose of PCBE (400 mg/kg b.w.) maintained ( $P < 0.05$ ) the serum markers as  $25.70 \pm 0.002$  mg/dl,  $2.23 \pm 0.19$  mg/dl and  $1.12 \pm 0.04$ , respectively. However, the dose containing only PCBE maintained ( $P < 0.05$ ) the serum markers concentration near to control.

### Effect of PCBE on antioxidant enzymes in kidneys of rat

A reduction in the level of CAT, POD, and SOD was observed in case of CCl<sub>4</sub> treatment (Table 3). The decline in antioxidant enzymes in CCl<sub>4</sub> treated animals were effectively ( $P < 0.05$ ) restored by PCBE. The values of CAT, POD, and SOD in CCl<sub>4</sub> treated animals were  $0.73 \pm 0.11$  U/min,  $1.26 \pm 0.21$  U/min and  $1.07 \pm 0.43$  U/mg proteins, respectively. When treated with low dose of PCBE the values of these enzymes were observed as  $1.90 \pm 0.24$  U/min,  $6.73 \pm 0.72$  U/min and  $1.57 \pm 0.32$  U/mg protein, respectively. The highest dose of PCBE increased the level, i.e.  $3.83 \pm 0.32$  U/min,  $8.56 \pm 1.11$  U/min and  $2.10 \pm 0.33$  U/mg protein, respectively, in comparison with control values. The dose containing only PCBE was also found to exhibit conditions almost near to the control values.

**Table 1.** Effect of PCBE on urine pH, specific gravity, leukocytes, RBC and albumin

Treatment	pH	Specific Gravity	Leukocytes/ $\mu$ l	RBC/ $\mu$ l	Albumin (mg/dl)
Control	$6.6 \pm 0.17b$	$1.05 \pm 0.14c$	$8.2 \pm 0.51b$	$0.25 \pm 0.06d$	$6.23 \pm 0.01d$
Olive oil + DMSO	$6.7 \pm 0.11b$	$1.07 \pm 0.13c$	$8.9 \pm 0.63b$	$0.37 \pm 0.07c$	$6.80 \pm 0.01c$
1 mL/kg b.w. CCl <sub>4</sub>	$5.6 \pm 0.21c$	$1.55 \pm 0.21a$	$16.8 \pm 0.24a$	$14.22 \pm 1.64a$	$13.26 \pm 0.00a$
Sily. + 1 mL/kg b.w. CCl <sub>4</sub>	$6.8 \pm 0.18ab$	$1.14 \pm 0.12bc$	$7.9 \pm 0.82c$	$0.45 \pm 0.13b$	$7.15 \pm 0.00c$
200 mg/kg b.w. PCBE + CCl <sub>4</sub>	$6.7 \pm 0.22b$	$1.06 \pm 0.13c$	$7.3 \pm 0.64c$	$0.53 \pm 0.12b$	$5.96 \pm 0.00d$
400 mg/kg b.w. PCBE + CCl <sub>4</sub>	$6.8 \pm 0.16ab$	$1.13 \pm 0.11bc$	$9.2 \pm 0.74b$	$0.37 \pm 0.14c$	$7.72 \pm 0.02b$
400 mg/kg b.w. PCBE alone	$7.2 \pm 0.01a$	$1.27 \pm 0.14b$	$9.2 \pm 0.92b$	$0.27 \pm 0.03d$	$5.87 \pm 0.00d$

Note: Mean  $\pm$  SD (n=3). Sily. (Silymarin), PCBE (*P. chinensis* bark ethanol extract); a-d (means with different letters) indicate significance at  $P < 0.05$

**Table 2.** Effect of PCBE on serum urea, creatinine, and bilirubin

Treatment	Urea (mg/dl)	Creatinine (mg/dl)	Bilirubin (mg/dl)
Control	$28.1 \pm 2.6b$	$0.35 \pm 0.07d$	$0.34 \pm 0.18b$
Olive oil + DMSO	$26.7 \pm 2.2b$	$0.42 \pm 0.12c$	$0.38 \pm 0.16bc$
1 mL/kg b.w. CCl <sub>4</sub>	$72.9 \pm 6.8a$	$1.42 \pm 0.25a$	$1.97 \pm 0.26a$
Sily. + 1 mL/kg b.w. CCl <sub>4</sub>	$27.4 \pm 2.3b$	$0.43 \pm 0.06c$	$0.84 \pm 0.27b$
200 mg/kg b.w. PCBE + CCl <sub>4</sub>	$23.3 \pm 2.3c$	$0.62 \pm 0.13b$	$0.92 \pm 0.15c$
400 mg/kg b.w. PCBE + CCl <sub>4</sub>	$25.7 \pm 2.2b$	$0.42 \pm 0.13c$	$0.72 \pm 0.24b$
400 mg/kg b.w. PCBE alone	$28.9 \pm 2.2b$	$0.38 \pm 0.08cd$	$0.37 \pm 0.16bc$

Note: Mean  $\pm$  SD (n=3). Sily. (Silymarin), PCBE (*P. chinensis* bark ethanol extract); a-d (means with different letters) indicate significance at  $P < 0.05$

**Table 3.** Effect of PCBE on antioxidant enzymes CAT, POD, and SOD of rat kidney

Treatment	CAT (U/min)	POD (U/min)	SOD (U/mg protein)
Control	$4.53 \pm 0.36a$	$8.66 \pm 1.71a$	$2.76 \pm 0.21a$
Olive oil + DMSO	$4.24 \pm 0.32a$	$7.99 \pm 1.61a$	$2.56 \pm 0.23a$
1 mL/kg CCl <sub>4</sub>	$0.73 \pm 0.11e$	$1.26 \pm 0.21c$	$1.07 \pm 0.43c$
Sily. + 1 mL/kg b.w. CCl <sub>4</sub>	$3.31 \pm 0.28c$	$7.46 \pm 1.12ab$	$2.46 \pm 0.52ab$
200 mg/kg b.w. PCBE + CCl <sub>4</sub>	$1.90 \pm 0.24d$	$6.73 \pm 0.72b$	$1.57 \pm 0.32c$
400 mg/kg b.w. PCBE + CCl <sub>4</sub>	$3.83 \pm 0.32bc$	$8.56 \pm 1.11a$	$2.10 \pm 0.33b$
400 mg/kg b.w. PCBE alone	$4.10 \pm 0.37a$	$8.13 \pm 1.22a$	$2.68 \pm 0.61a$

Note: Mean  $\pm$  SD (n=3). Sily. (Silymarin), PCBE (*P. chinensis* bark ethanol extract); a-e (means with different letters) indicate significance at  $P < 0.05$

**Table 4.** Effect of PCBE on antioxidant enzymes GSH-Px, GSR, GST, QR and  $\gamma$ -GT of rat kidney

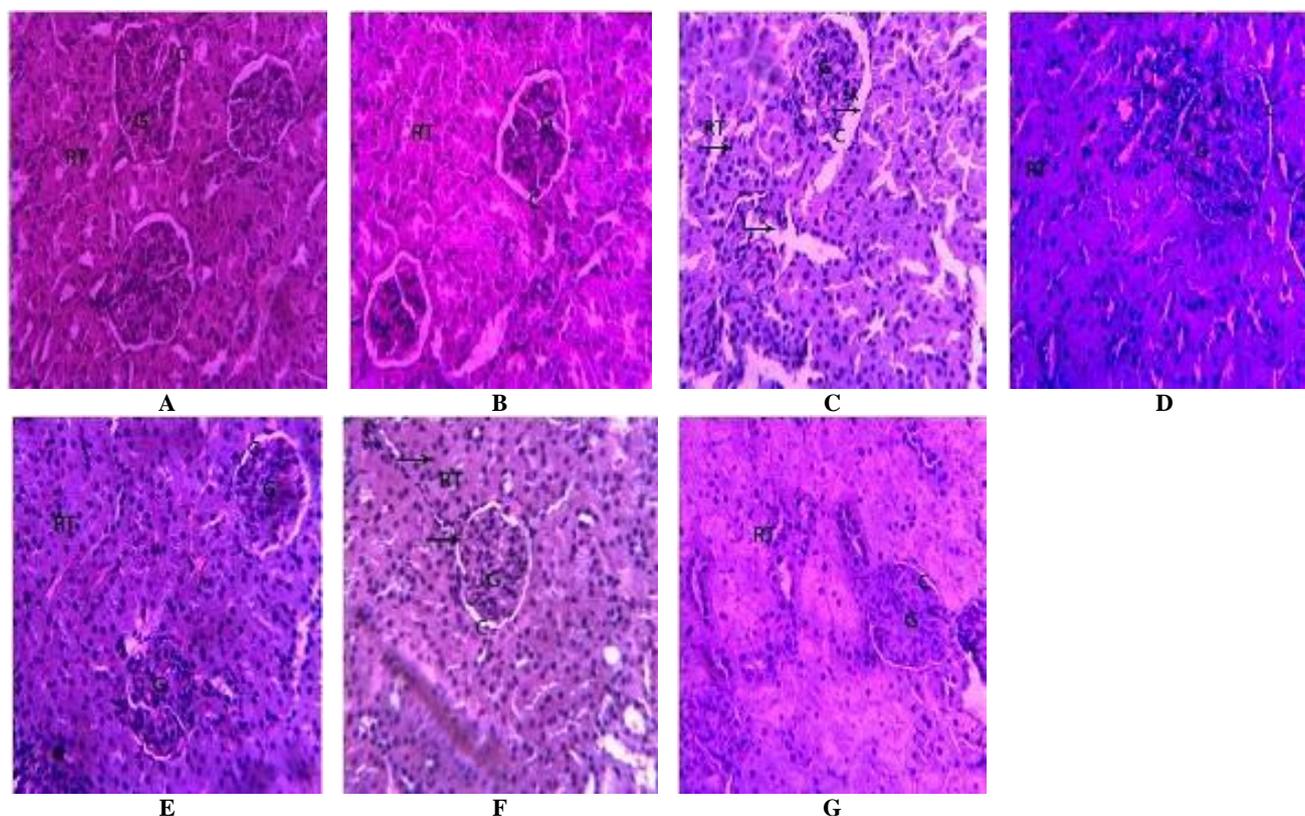
Treatment	GSH-Px (nM/min/mg protein)	GSR (nM/min/mg protein)	GST (nM/min/mg protein)	QR (nM/min/mg protein)	$\gamma$ -GT (nM/min/mg protein)
Control	134.0±8.5a	175.7±8.2a	208.3±12.2ab	118.0±8.4b	402.7±16.2a
Olive oil + DMSO	132.1±8.2a	177.2±9.2a	225.0±13.3a	120.1±7.3ab	415.0±13.9a
1 mL/kg b.w. CCl <sub>4</sub>	75.7±4.2c	93.0±5.4d	107.3±7.2d	65.3±4.8d	102.0±8.4d
Sily + 1 mL/kg b.w. CCl <sub>4</sub>	135.0±6.4a	186.7±10.2a	224.7±12.2a	132.0±6.4a	375.0±12.5b
200 mg/kg b.w. PCBE + CCl <sub>4</sub>	99.0±6.4b	115.7±7.1c	169.7±11.7c	99.0±7.4c	108.6±7.2d
400 mg/kg b.w. PCBE + CCl <sub>4</sub>	125.0±7.4a	164.0±8.4b	200.0±10.4b	135.7±9.3a	124.7±6.2d
400 mg/kg b.w. PCBE alone	130.1±7.1a	171.2±9.3ab	222.1±11.2ab	134.0±7.3a	301.0±9.4ab

Note: Mean  $\pm$  SD (n=3). Sily. (Silymarin), PCBE (*Pistacia chinensis* bark ethanol extract); a-e (means with different letters) indicate significance at P<0.05

**Table 5.** Effect of PCBE on the tissue proteins, antioxidant enzymes TBARS, H<sub>2</sub>O<sub>2</sub> and nitrite contents in rat kidney

Treatment	Protein $\mu$ g/mg tissue	GSH ( $\mu$ M/g tissue)	TBARS nM/min /mg protein	H <sub>2</sub> O <sub>2</sub> $\mu$ M/ml	Nitrite $\mu$ M/ml
Control	2.97±0.47a	21.76±3.2a	1.51±0.26c	4.5±0.31c	58.22±4.11c
Olive oil + DMSO	3.08±0.35a	19.88±2.8a	1.14±0.16b	4.5±0.24c	58.88±3.58c
1 mL/kg b.w. CCl <sub>4</sub>	1.67±0.36c	5.86±0.62c	4.3±0.22a	12.8±1.32a	97.07±6.62a
Sily. + 1 mL/kg b.w. CCl <sub>4</sub>	2.85±0.48ab	19.26±2.2a	2.34±0.11b	6.5±0.82c	57.99±3.22c
200 mg/kg b.w. PCBE + CCl <sub>4</sub>	2.65±0.58b	11.90±1.4b	3.81±0.16d	7.5±0.64b	67.88±3.24b
400 mg/kg b.w. PCBE + CCl <sub>4</sub>	3.02±0.37a	17.83±2.3a	2.15±0.18cd	5.5±0.45bc	58.12±2.75c
400 mg/kg b.w. PCBE alone	3.15±0.36a	20.54±2.3a	1.26±0.21b	4.3±0.42c	57.48±3.22c

Note: Mean  $\pm$  SD (n=3). Sily. (Silymarin), PCBE (*P. chinensis* bark ethanol extract); a-d (means with different letters) indicate significance at P<0.05



**Figure 1.** Histopathological architecture of rat kidney (A) Control, (B) DMSO + Olive oil, (C) CCl<sub>4</sub> treated group, (D) CCl<sub>4</sub> + Silymarin, (E) PCBE (200 mg/k.g. b.w.) + CCl<sub>4</sub>, (F) PCBE (400 mg/k.g. b.w.) + CCl<sub>4</sub>, (G) PCBE only. (RT) Renal tubules, (G) Glomerulus, (C) Bowman's capsule, (^) Increase of space between capsule and glomerulus, (\*) Glomerulus atrophy.

Naturally existing metabolic system helps in the reduction of oxidative injury in various organs. As a result of metabolism, various hazardous metabolites are formed. In a cell or tissue, many anti-oxidant enzymes are involved in the reduction of such injurious products in a scavenging process. The restoration of decrease in the activity of GSH-Px, GSR, GST, QR, and  $\gamma$ -GT enzymatic values was remarkably ( $P < 0.05$ ) achieved by PCBE treatment (Table 4). A significant decrease ( $P < 0.05$ ) in the values of GSH-Px, GSR, GST, QR, and  $\gamma$ -GT was observed in  $CCl_4$  treated rats. The high and low dose of PCBE were found to increase ( $P < 0.05$ ) the level of these antioxidant enzymes as compared to the  $CCl_4$  treated animals. Whereas, the dose containing only PCBE showed all the enzymatic levels of tissue almost near to the control.

#### **Effect of PCBE on biochemical parameters in kidneys of rat**

The effect of PCBE on tissue proteins, TBARS,  $H_2O_2$  and nitrite content of renal samples was also evaluated. In  $CCl_4$  treated animals a decrease in tissue proteins and reduced glutathione content whereas, an increase in TBARS,  $H_2O_2$  and nitrite content was observed (Table 5). Restoration of these values was observed in animals given PCBE. More ameliorated effects on  $CCl_4$  induced toxicity was recorded with higher dose on these parameters. However, animals administered only PCBE displayed results near to control.

#### **Histopathology of rat kidney**

Histoarchitecture of rat kidneys treated with different doses is described in Figure 1 A-G. The intraperitoneal injection of  $CCl_4$  caused high damage to the cortex region of kidney. Obvious damage to the glomerulus and outer Bowman's capsule of kidney nephrons was observed in  $CCl_4$  treated organism. Increased space between the capsule and the glomerulus was detected with dilation of renal tubules of kidney. High disruption of glomerulus and inflammation of the cells of both medulla and cortex was clearly visible. The low dose of PCBE (200 mg/kg. b.w.) had minimized the chronic damages but the high dose of PCBE (400 mg/kg. b.w.) showed notable restoration of tissue structure with a minor dilation of capillary tubules. DMSO and control groups had normal histological structure. While the silymarin treated group also showed normal histological architecture.

#### **Discussion**

Traditional herbs derived medicines are in use as a remedy of various diseases from ancient times, about more than 5,000 years back. The cause of various diseases is the proliferation of free radicals in the cell and the plant associated polyphenols are identified to be highly capable of trapping such free radicals, this ability of plants has enhanced attention of researchers towards the use of traditional medicines. Flavonoids and phenols are chief constituents of plants due to their antioxidant nature. Fruits, vegetables, seeds, fruits, leaves, bark, roots, nuts and other

parts of plants are a rich source of plant phenolics (Kaviarasan et al. 2007).

Xenobiotics are toxic chemicals such as  $CCl_4$ , which triggers the generation of ROS in a cell and cause damage to kidney tissues. The effect of  $CCl_4$  on kidney is found to be higher than other organs (Sanzgiri et al. 1997). Abraham et al. (1999) and Stacchiotti et al. (2002) reported high affinity of kidney cells towards  $CCl_4$ , as cytochrome P450 presence is abundant in its cortex.

Kidneys exist in a paired system in all mammals and are associated with the discharge of toxic metabolic products and other wastes. For the removal of the wastes from blood, kidney nephrons take in and pass on a vast amount of fluid. A highly concentrated amount of such wastes is removed outside the body to retain body fluid level (Hickey et al. 2001). Currently, pathogenesis of kidneys is becoming an alarming issue regarding public health, as the key role of kidneys in the regulation of diverse toxins and chemicals is now well studied (Ozturk et al. 2003).

The ability of phenolics to carry out redox reactions implicates its antioxidant properties, involving in the scavenging of free radicals, quenching free oxygen and complex formation with pro-oxidant metals (Mustafa et al. 2010). Polyphenols are widely studied bioactive compounds and are known to maintain a number of biological processes. Polyphenols are abundantly distributed among the plants and in a substantial amount is been ingested by the humans. Their anti-mutagenic properties, anti-cancerous effect and protective ability against certain chemicals have made them highly venerable for extensive research (Brown 1980).

This study was carried out to evaluate the nephroprotective effect of *P. chinensis* in the animals treated with  $CCl_4$ , which is a potent nephrotoxin. Its protective effect was evident from the results obtained and was found to be highly proficient in scavenging of free radicals. All of the enzymatic and non-enzymatic antioxidants activity was observed to be retained under the influence of *P. chinensis*.

According to Simerville et al. (2005), the venerable means to account disruptions associated with urinary system is the analysis of urine. In the experimental analysis, the parameters of urine profile were examined in the first place, i.e. pH, specific gravity, RBCs, pus cells, albumin. A reduction in urine pH and increase in all other parameters was observed in  $CCl_4$  treated animals. The antioxidant ability of *P. chinensis* was obvious by the maintenance of the normal values for all parameters.

It was reported that the administration of  $CCl_4$  causes a high degree of oxidative damage to proteins and lipids of kidney in rats (Khan et al. 2009). Carbon tetrachloride administration results in nephrotoxicity which can be spotted by the sharp alterations in the urine and serum levels of creatinine, urobilinogen, creatinine clearance, and urea. Such pathological variations indicate the impending impairments in kidneys treated with  $CCl_4$  (Ogeturk et al. 2005). In a study by Khan and Zehra (2013) altered urea level in blood and severe damage to kidney tissues was

reported. The high levels of creatinine, albumin, and proteins also indicate chronic renal damage. In our study, a notable decrease in the concentration of urea and creatinine, while an increase in bilirubin level was observed in CCl<sub>4</sub> treated animals, this raised level of bilirubin evidently indicated the high breakdown of RBCs. The *P. chinensis* treatment was testified to normalize all the conditions without any side effect.

According to Khan et al. (2009) catalase (CAT), superoxide dismutase (SOD) and peroxidase (POD) establish a cascade of defense mechanism against the stress caused by reactive oxygen species. Such oxidative stress results in the reduction of these antioxidant enzymes and makes the cell vulnerable to great damage by lessening its antioxidant status. The antioxidant SOD is functioned to remove superoxides by transforming these radicals to hydrogen peroxide (Halliwell and Gutteridge 2007). Catalase (CAT) is a hemoprotein, which plays its role in the protection of tissue from exogenous oxidative stress by scavenging hydrogen peroxide in a cell (Sahreem et al. 2010).

At molecular level, first line of defense is established by glutathione which maintains cell integrity by scavenging free radicals. The reduction of GSH level in a tissue can be the result of NADPH decrease or GSH consumption in the removal of peroxides (Yadav et al. 1997). GSH-dependent enzymes provide the second defense line; predominantly involved in the detoxification of lethal ROS generated byproducts and extermination of free radicals (Gumieniczek 2005). The peroxides are detoxified by the reaction of GSH-Px with GSH to form GSSG, which is further reduced by glutathione reductase (GSR) to glutathione (GSH) (Maritim et al. 2003). The peroxidation of polyunsaturated fatty acids finally leads to the formation of TBARS; the final metabolites of this series of reactions and are considered as late biomarkers of oxidative stress (Khan and Zehra 2013).

Ji et al. (1988) reported the reduced levels of GSR, GSH-Px, SOD, CAT, GR, and GST as the result of CCl<sub>4</sub> induced stress in treated animals. These antioxidants are part of cell defense mechanism and any hindrance in their activity means loss of stability in cell due to the ROS formation. (Khan et al. 2009) also reported the decrease in the level of these antioxidants. In present study, a prominent decline in the levels of CAT, POD, SOD, GSH, GSH-Px, GSR, GST, QRT,  $\gamma$ -GT whereas, a rise in lipid peroxidation, H<sub>2</sub>O<sub>2</sub> and nitrite content was noticeable in CCl<sub>4</sub> treated rats. *P. chinensis* was detected to rise up the concentration of all these antioxidants with reduction in the lipid peroxidation. Thus, retaining the cell stability and functioning. This study was in harmony with (Khan and Siddique 2012; Moneim and El-Deib 2012; Khan and Zehra 2013).

CCl<sub>4</sub> induction showed the inflammation and atrophy of kidney glomeruli, shortening of epithelial cells lining in the surrounding tubules and diminishing of sharp edges (Moneim and El-Deib 2012). Other morphological changes include interstitial fibrosis and cellular infiltration (Ozturk et al. 2003; Jayakumar et al. 2008). Adewole et al. (2007) also reported the kidney cells histopathological damages

involving congestion of tubules due to the overloading of kidney nephrons. Our study showed results accordingly, a clear disruption of kidney glomeruli, increased distance between the capsule and glomeruli was observed besides occurrence of swollen proximal and distal tubules in CCl<sub>4</sub> treated animals. Whereas, the administration of *P. chinensis* bark extract retained the normal conditions and reduced the toxic effect of CCl<sub>4</sub>. These results were in coherence with (Khan et al. 2009), who reported that urea and creatinine rise in serum was associated with chronic renal damages by CCl<sub>4</sub> intoxication and are considered as indicators of kidney cellular disruptions, where the serum creatinine level does not rise until at least half of the kidney nephrons are destroyed. Renal injuries may contribute to low level of serum protein that might have resulted from remarkable leakage into urine due to injuries in glomeruli and tubules (Khan et al. 2011).

## CONFLICT OF INTEREST

The authors declare that no conflict of interest.

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