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Research Article
Protective Effect of *Nicotiana plumbaginifolia* Linn against CCl_4 Induced Oxidative Stress in *Gallus gallusdomesticus*

[1,2]Abdus Saboor Shah, [1]Mushtaq Ahmed, [3]Noor ul Haq and [1]Rahmat Ali Khan

[1]Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology Bannu, 2800, Khyber-Pakhtunkhwa, Pakistan
[2]Department of Chemistry, Govt. Post graduate College, Bannu, Khyber-Pakhtunkhwa, Pakistan
[3]Department of Computer Science and Bioinformatics, Khushal Khan Khattak University, Karak, 27200, Khyber-Pakhtunkhwa, Pakistan

Abstract

Background and Objective: Oxidative stress that may be the source of different disorders is caused by reactive oxygen species. CCl_4 may cause oxidative stress. Antioxidant enzymes that are created by the body may scavenge the reactive oxygen species. The protective effects of *Nicotiana plumbaginifolia* (Linn) methanolic extract (NPME) against CCl_4 induced oxidative damage in the kidneys and lungs of chicks were determined in this study. **Materials and Methods:** Two months old male *Gallus gallusdomesticus* chicks were subjected to this experiment. Total 36 chicks were divided into six groups i.e., Group I to VI were subjected under control, DMSO (1 mL kg^{-1} b.wt.), 1 mL kg^{-1} b.wt. CCl_4 (50% in olive oil), silymarin (50 mg kg^{-1} b.wt.) after 24 hrs of CCl_4 administration, 100 and 200 mg kg^{-1} b.wt. NPME after 24 hrs of CCl_4 administration for three weeks. **Results:** The results revealed that CCl_4 induced oxidative stress was more pronounced in the kidneys as compared to the lungs. CCl_4 caused a significant decrease in the activity of kidney antioxidant enzymes (CAT, POD, GSH-Px) and glutathione (GSH), while an increase in the levels of TBARS, creatinine and urea than that of lungs samples. **Conclusion:** Since NPME treatment recovered towards normal conditions directly proportional to the dose applied and thus it was concluded that *Nicotiana plumbaginifolia* (Linn) methanolic extract can protect the kidneys more significantly than lungs tissues against CCl_4 oxidative stress.

Key words: *Nicotiana plumbaginifolia*, oxidative stress, CCl_4, silymarin, glutathione, creatinine

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Corresponding Author: Rahmat Ali Khan, Department of Biotechnology, Faculty of Biological Sciences, University of Science and Technology Bannu, 2800, Khyber-Pakhtunkhwa, Pakistan

Competing Interest: The authors have declared that no competing interest exists.

Data Availability: All relevant data are within the paper and its supporting information files.

INTRODUCTION

Reactive oxygen species (ROS e.g. superoxide radicals (O^-) and hydroxyl radicals (OH^-) are produced under normal metabolic conditions as by products[1-2]. Different conditions like exposure to high temperature, radiations, smoking and heavy metals can induce the production of reactive oxygen species which causes oxidative stress[3]. Intoxication of carbon tetrachloride (CCl_4) in animals causes oxidative stress[4]. Different studies have revealed that free radicals are generated in kidneys, lungs, heart and blood cells under CCl_4 intoxication[5]. CCl_4 is a synthetic chemical that damages liver and kidney cells[6] because it increases triburbitoric acid and decreases the activity of antioxidant enzymes[6-8]. CCl_4 is bioactivated by cytochrome P450 system and converts into very reactive trichloromethyl CCl_3• radical which in the presence of oxygen form the more toxic trichloromethyl peroxyl radical (CCl_3O_2•)[6]. These free radicals then bind with fatty acid-producing alkoxy (R•) and peroxy radicals (ROO•) which creates highly reactive lipid peroxides that bring changes in the enzyme activity and stimulate injury[8-9]. Researchers have suggested that trichloromethyl (CCl_3•) free radicals combine with SH groups of glutathione (GSH) and proteins. This binding of trichloromethyl free radicals to cellular proteins initiates membrane lipid peroxidation, apoptosis and finally cell necrosis[10-11]. Besides the antioxidant enzymes, CCl_4 also changes the biochemistry of the kidney such as serum creatinine, albumins and bilirubins are changed[12]. It is reported in the literature that various herbal extracts have a counter effect against CCl_4 induced oxidative stress by changing the levels of lipid peroxidation and induce the activities of antioxidant enzymes. It also regulates the decreased level of GSH[13]. CCl_4 causes injury to the lungs[14-15]. The injuries are induced by CCl_4 in different organs through oxidative stress which causes lipid peroxidation and lower the endogenous antioxidant enzymes[16-17]. Asthma, chronic obstructive pulmonary disease and cystic fibrosis are common diseases of lungs in which the production of reactive oxygen species is increased[18-19]. It has also been reported in the literature that asthma reduces glutathione peroxidase and superoxide dismutase[20]. It has also been observed that oxidative stress is the main cause of numerous disorders and diseases which can only be diminished through antioxidants to maintain human health[21].

Since synthetic antioxidants result in numerous side effects therefore it is necessary to investigate natural antioxidants with fewer side effects and large efficacy. *Nicotiana plumbaginifolia* has been reported with rutin quercetin and alkaloids which are good antioxidants. *Nicotiana plumbaginifolia* has traditionally been used to treat several ailments from ancient time. The present study has been conducted to evaluate the protective effect of the crude effect of *Nicotiana plumbaginifolia* in kidneys and lungs tissue against CCl_4 intoxication in chicks.

MATERIALS AND METHODS

This work has been completed in the Department of Biotechnology, University of Science and Technology, Bannu, Khyber-Pakhtunkhwa, Pakistan. All the experimentation was completed in six months (July to December, 2017). The plant's collection and drying were completed in one month while the chicks' acclimatization and dose administration were continued for four weeks. The details were given in the experimental design section.

Plant collection: *Nicotiana plumbaginifolia* (Linn) plants were collected from Bannu, Khyber-Pakhtunkhwa, Pakistan and were identified by Prof. Abdurrahman, Department of Botany, Government Post Graduate College, Bannu, Khyber-Pakhtunkhwa, Pakistan. The plant samples were washed using de-ionized water and were shade dried at room temperature for 25 days followed by chopping and grinding mechanically to bring it into a powder form as described by Shah *et al.*[22].

Preparation of crude extract: *Nicotiana plumbaginifolia* (Linn) plant powder (200 g) was socked in 1 L of 80% methanol and shaken randomly for seven days. After seven days, the plant extract was filtered using Whatman filter paper No. 1 and then concentrated with the help of a rotary evaporator under reduced pressure at 37°C. This crude extract was then stored at 4°C for further investigation.

Experimental design: To investigate the antioxidant potential of *Nicotiana plumbaginifolia in vivo*, 36 male chicks were equally divided into 6 groups. Group I chicks were kept under control conditions and group II was provided with DMSO (1 mL kg^{-1} b.wt.), group III was treated with 1 mL kg^{-1} b.wt. CCl_4 (50% in olive oil), group IV was provided with silymarin (50 mg kg^{-1} b.wt.) after 24 hrs of CCl_4 administration while group V and VI chicks were treated with NPME (100 and 200 mg kg^{-1} b.wt.) after 24 hrs of CCl_4 administration respectively for three weeks. After 24 hrs of last treatment, blood was collected from the wings vein and centrifuged at 6,000×g for serum separation. All the chicks were slaughtered and removed from their kidneys as well as lungs followed by weighing and keeping in 10% formalin and stored at -20°C. Half of the kidneys and lungs tissues were subjected for further enzymatic analysis.

Assessment of antioxidant enzymes: Kidneys and lungs tissues (200 mg) were homogenized in 10 volumes of PO_4 buffer (pH 7.4) and centrifuged at 6,000×g for 30 min. The supernatant was collected and used for enzymatic, protein and TBARS assays. Protein concentration of the supernatant of kidneys and lungs tissues was determined using the method demonstrated in Lowry *et al.*[23] and crystalline Bovine Serum Albumin (BSA) was used as a standard.

Catalase assay (CAT): Catalase (CAT) activities were determined by the protocol presented in Manna *et al.*[24] with some modifications. The reaction mixture (2.2 mL) containing 2 mL, 50 mM phosphate buffer (pH 5.0), 0.1 mL of 5.9 mM hydrogen peroxide (H_2O_2) and 0.1 mL enzyme extract solution was used for absorbance measurement. Changes in absorbance of the reaction solution at 420 nm were determined after 30 sec. One unit of CAT activity was defined as an absorbance change of 0.01 units min^{-1}.

Peroxidase assay (POD): Activities of POD were determined by the method already reported[25] with some modifications. The Peroxidase (POD) reaction mixture (2.5mL) was prepared using 50 mM phosphate buffer (pH 5.0), 0.1 mL of 20 mM guaiacol, 0.3 mL of 40 mM hydrogen peroxide (H_2O_2) and 0.1 mL enzyme extract. Changes in absorbance of the reaction mixture were noted at 470 nm after 1 min. One unit of POD activity was defined as an absorbance change of 0.01 as unit/min.

Glutathione reductase assay (GSH): Carlberg and Mannervik[26] were followed to assess the glutathione reductase activity. Assay mixture (2 mL) was consisting of 0.1 mL Post Mitochondrial Supernatant (PMS), 1.65 mL phosphate buffer (0.1 molar; pH 7.6), 0.1 mL ethylene diamine tetraacetate (EDTA), 0.05 mL oxidized glutathione (1 mM) and 0.1 mL NADPH (0.1 mM). The enzyme activity was noted at 25°C by measuring the disappearance of NADPH at 340 nm and was calculated as nmol oxidized/min mg protein using a molar extinction coefficient of 6.22×10^3/M cm^{-1}.

Glutathione peroxidase assay (GSH-Px): Glutathione peroxidase (GSH-Px) activity was measured by protocol presented in Mohandas *et al.*[27]. The reaction mixture (2 mL) was prepared using 1.49 mL PO_4 buffer (0.1 M; pH 7.4), 1 mM EDTA (0.1 mL), 1 mM sodium azide NaN_3 (0.1 mL), 0.05 mL glutathione reductase (1 IU mL^{-1}), 1 mM GSH (0.05 mL), 0.2 mM NADPH (0.1 mL), 0.25 mM H_2O_2 (0.01 mL) and 0.1 mL of tissues homogenate. The disappearance of NADPH at 340 nm was noted. Enzyme activity was calculated as nmol NADPH oxidized/min/mg protein using molar extinction coefficient of 6.22×10^3 M cm^{-1}.

Glutathione-S-transferase assay (GST): This activity was determined using the procedure presented in Habig *et al.*[28]. The reaction mixture (2 mL) was prepared by mixing 1.475 mL phosphate buffer (0.1 M, pH 6.5), 0.2 mL (200 µL) glutathione reduced (1 mM), 0.025 mL CDNB (1 mM) and 300 µL tissue homogenate. The changes in the absorbance were recorded at 340 nm and enzyme activity was calculated as nM CDNB conjugate formed/min/mg protein using a molar extinction coefficient of 9.6×10^3 M cm^{-1}.

Protein estimation: The total soluble proteins in the homogenate of kidneys and lungs tissues were estimated by the method described in Lowry *et al.*[23]. BSA standard curve was used as standard. The amount of protein was expressed as mg mL^{-1} of tissue homogenate.

Lipid peroxidation assay: Wright *et al.*[29] was followed for lipid peroxidation. The reaction solution (1.0 mL) contained 0.58 mL PO_4 (0.1 M, pH 7.4), 0.2 mL homogenate sample, 0.2 mL ascorbic acid (100 mM) and 0.02 mL ferric chloride (100 mM) solution. The reaction mixture was incubated at 37°C for 60 min followed by the addition of 1.0 mL trichloroacetic acid (10%). Following the addition of 1.0 mL 0.67% thiobarbituric acid (TBA), tubes were placed in a boiling water bath for 25 min and then shifted to crushed ice-bath before centrifuging at 3000×g for 10 min. The amount of TBARS formed in each sample was calculated by measuring the absorbance of the supernatant at 535 nm. The results were expressed as nmol TBARS/min/mg tissue at 37°C using a molar extinction coefficient of 1.56×10^5 M cm^{-1}.

Serum biochemistry: Blood was collected from the veins of the chicks just before the slaughtering stage of the experiment. Blood samples were centrifuged and serum was stocked up at -20°C. Samples were subjected to analyze the serum albumin, creatinine and urea AMP using diagnostic kits.

Statistical analysis: Data have been presented as mean standard deviation. Computer software (SPSS version 16) was used for statistical analysis. The analysis of variance and post hoc multiple comparison tests were performed to estimate the differences among the different groups. The value of $p<0.05$ was considered significant.

RESULTS

Effect of NPME on proteins and antioxidant enzymes of kidney and lungs: Level of antioxidant enzymes (CAT and POD) in the kidney reduced under CCl_4 intoxication as shown in Table 1. The data showed a significant decrease as compared to the control condition samples. These enzymes showed a minimum level under CCl_4 while under NPME (200 mg kg^{-1} b.wt.), these showed a significant increase in the silymarin group. Dose-dependent treatment of NPME showed a significant effect. The same pattern was observed while studying the antioxidant activity of lungs homogenate but comparatively low alteration as shown in Table 2.

Effect of NPME on GSH-Px and GST activities: Alteration in kidney and lungs GSH-Px and GST have been shown in Table 3 and 4. GST and GSH-Px activities are significantly reduced under CCl_4 treatment as compared to the control conditions samples. Treatment with NPME in dose-dependent manner and silymarin markedly increased the levels of GSH-Px and GST. However, the effect of NPME is greater in kidneys than that of lungs tissues.

Effects of NPME on GSH and TBARS contents: Glutathione reductase (GSH) played a significant role in the metabolism of free radicals and tries to reduce triberbitoric acid reactive substances (TBARS). Administration of CCl_4 reduced GSH contents and increased TBARS formation in kidneys and lungs as shown in Table 5 and 6. Supplementation of NPME reversed the contents and protects cells from lipid peroxidation. NPME @ 200 mg kg^{-1} showed a great effect in kidneys as that of silymarin while less efficacy in lungs.

Effect of NPME on body and tissues weight of chicks: Data recorded in Table 7 indicated the effect of NPME on the body and tissues weight of chicks. Group intoxicated with CCl_4 has shown a significant decrease in absolute and per cent weight of the whole body while the absolute, as well as relative weight of the kidneys and lungs, were increased under the formerly mentioned treatment. Treatment of CCl_4-treated chicks with different concentrations of NPME restored the absolute and percentage body weight as well as absolute and relative kidney weight. However, the effect of NPME was dose-dependent and more pronounced in the kidney as compared to the lungs.

Effect of NPME on serum profile in chicks: The serum concentration of creatinine, albumin and urea shows the level of the injuries in the kidney. The effect of CCl_4 on the above-mentioned factors was presented in Table 8. Treatment of CCl_4 significantly increased the serum level of creatinine and urea while albumin was reduced as compared to the control conditions samples. The toxicity of CCl_4 and the level of serum creatinine, albumin and urea were normalized under different doses of NPME.

Table 1: Effect of NPME on the protein and antioxidant enzymes in kidney of chicks

Group treatment	Protein (μg mg^{-1} tissue)	CAT (U min^{-1})	POD (U min^{-1})
Control	460±6.87^{++}	0.80±0.03^{++}	0.70±0.05^{++}
DMSO	436±11.90^{++}	0.70±0.02^{++}	0.80±0.02^{++}
CCl_4	365±8.12**	0.30±0.05**	0.10±0.04**
CCl_4+silymarin 50 mg kg^{-1} b.wt.	423±5.63^{++}	0.50±0.04^{++}	0.40±0.05^{++}
CCl_4+NPME 100 mg kg^{-1} b.wt.	386±7.02*$^{+}$	0.40±0.03*$^{+}$	0.50±0.06^{++}
CCl_4+NPME 200 mg kg^{-1} b.wt.	405±4.09^{++}	0.60±0.01^{++}	0.66±0.06^{++}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

Table 2: Effect of NPME on the protein and antioxidant enzymes in lungs of chicks

Group treatment	Protein (μg mg^{-1} tissue)	CAT (U min^{-1})	POD (U min^{-1})
Control	385±12.02^{++}	0.75±0.05^{++}	0.73±0.07^{++}
DMSO	379±5.09^{++}	0.70±0.04^{++}	0.78±0.05^{++}
CCl_4	211±7.04**	0.50±0.04**	0.30±0.03**
CCl_4+silymarin 50 mg kg^{-1} b.wt.	316±4.43^{++}	0.55±0.09	0.42±0.04^{+}
CCl_4+NPME 100 mg kg^{-1} b.wt.	304±5.76^{++}	0.51±0.07	0.39±0.05
CCl_4+NPME 200 mg kg^{-1} b.wt.	345±7.32^{++}	0.62±0.08^{+}	0.56±0.02^{+}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

Table 3: Effect of NPME on GSH-Px and GST in chicks kidney

Group treatment	GST (nmol/min/mg protein)	GSH Px (mol g^{-1} tissue)
Control	48.57±3.45^{++}	147.04±14.6^{++}
DMSO	46.06±4.32^{++}	142.6±12.4^{++}
CCl_4	24.52±2.45**	68.16±8.21**
CCl_4+silymarin 50 mg kg^{-1} b.wt.	44.66±5.12^{++}	132.3±10.23^{++}
CCl_4+NPME 100 mg kg^{-1} b.wt.	28.10±2.32	95.44±7.12^{++}
CCl_4+NPME 200 mg kg^{-1} b.wt.	33.7±3.11^{+}	118.9±7.54^{++}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

Table 4: Effect of NPME on GSH-Px and GST in chicks lungs

Group treatment	GST (nmol/min/mg protein)	GSH Px (mol g^{-1} tissue)
Control	56.71±4.67^{++}	121.59±12.34^{++}
DMSO	57.30±5.43^{++}	124.03±10.09^{++}
CCl_4	38.67±3.56**	87.31±7.23*
CCl_4+silymarin 50 mg kg^{-1} b.wt.	49.88±6.12^{++}	97.97±6.32^{++}
CCl_4+NPME 100 mg kg^{-1} b.wt.	46.90±3.45^{++}	94.96±7.12^{++}
CCl_4+NPME 200 mg kg^{-1} b.wt.	51.03±4.56^{++}	107.04±7.09^{+}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

Table 5: Effects of NPME on GSH and TBARS in chicks kidney

Group treatment	GSH (mol g^{-1} tissue)	TBARS (nmol/min/mg protein)
Control	35.70±3.67^{++}	2.60±0.09^{++}
DMSO	35.20±4.09^{++}	2.07±0.07^{++}
CCl_4	11.35±0.78**	7.28±1.2**
CCl_4+silymarin 50 mg kg^{-1} b.wt.	31.57±1.89^{++}	3.12±0.9^{++}
CCl_4+NPME 100 mg kg^{-1} b.wt.	16.67±2.12^{++}	5.95±0.6^{++}
CCl_4+NPME 200 mg kg^{-1} b.wt.	25.5±2.21^{++}	4.68±0.7^{++}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

Table 6: Effects of NPME on GSH and TBARS in chicks lungs

Group treatment	GSH (mol g^{-1} tissue)	TBARS (nmol/min/mg protein)
Control	49.37±3.12^{++}	3.76±0.3^{++}
DMSO	51. 08±5.10^{++}	3.37±0.7^{++}
CCl_4	29.49±4.12**	5.90±0.9*
CCl_4+silymarin 50 mg kg^{-1} b.wt.	41.09±3.21^{+}	4.10±0.3^{+}
CCl_4+NPME 100 mg kg^{-1} b.wt.	32.67±2.76	4.96±0.6
CCl_4+NPME 200 mg kg^{-1} b.wt.	39.75±2.31^{+}	4.07±0.7^{+}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

Table 7: Effect of NPME on body and kidney weight of chicks

Group treatment	Increase b.wt. (%)	Absolute kidney weight (g)	Lungs weight (g)
Control	17.14±1.34^{++}	5.1±1.21^{++}	6.5±0.03^{++}
DMSO	15.74±1.21^{++}	5.2±0.98^{++}	5.9±0.05^{++}
CCl_4	10.56±0.98*	3.5±0.21*	4.2±0.01*
CCl_4+silymarin 50 mg kg^{-1} b.wt.	14.78±2.34^{++}	4.4±0.23^{+}	5.2±0.02^{+}
CCl_4+NPME 100 mg kg^{-1} b.wt.	12.76±3.21^{+}	3.9±0.23^{+}	5.5±0.05^{+}
CCl_4+NPME 200 mg kg^{-1} b.wt.	13.56±1.23^{+}	4.5±0.31^{+}	6.0±0.07^{++}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{+}$Significance from the CCl_4 group at p<0.05 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

Table 8: Effect of NPME on creatinine, albumin and urea

Group treatment	Creatinine (mg dL^{-1})	Albumin (mg dL^{-1})	Urea (mg dL^{-1})
Control	62.5±4.56^{++}	23.04±1.09^{++}	58.80±2.31^{++}
DMSO	61.3±7.12^{++}	24.20±1.89^{++}	59.03±3.21^{++}
CCl_4	79.09±8.23**	13.01±1.21**	78.92±3.09**
CCl_4+silymarin 50 mg kg^{-1} b.wt.	66.7±7.10^{++}	22.61±1.09^{++}	62.42±2.12^{++}
CCl_4+NPME 100 mg kg^{-1} b.wt.	67.2±5.89^{++}	19.07±1.21^{++}	64.63±2.89^{++}
CCl_4+NPME 200 mg kg^{-1} b.wt.	63.01±9.02^{++}	21.35±0.98^{++}	61.45±0.98^{++}

Mean±SE (n = 6 number). **Significance from the control group at p<0.01 probability level, $^{++}$Significance from the CCl_4 group at p<0.01 probability level

DISCUSSION

Researchers are focusing on the importance of plant-based chemo prevention against different diseases and ailments because of its low side effects and high efficacy. Different drugs and chemicals are the cause of the production of Reactive Oxygen Species (ROS). CCl_4 is a well-known chemical that stimulates ROS production, causes oxidative stress and reduces antioxidant defense. Here in this study, carbon tetrachloride (CCl_4) significantly reduced the glutathione Reductase (GSH) which is an antioxidant enzyme in kidneys. Additional to the above, catalase (CAT), glutathione S transferase and Glutathione peroxidase also showed decreased level under carbon tetrachloride (CCl_4) which shows oxidative stress. CCl_4 application may inhibit the non-enzymatic and enzymatic antioxidant defense systems in kidneys and thus glutathione Reductase (GSH) level has been reduced. This decrease may increase the generation of reactive oxygen species. The same results have been reported earlier[6,12].

Carbon tetrachloride (CCl_4) through reductive dehalogenation in the presence of the P450 enzyme system, generates the highly reactive trichloromethyl (CCl_3) radical which causes lipid peroxidation and ultimately the foundation of renal damage[12].

The high level of lipid peroxidation in the kidneys and lungs of the chicks under CCl_4 indicates overproduction of reactive oxygen species and its accumulation that result in dysfunction of antioxidants under CCl_4 conditions. NPME application results in a decrease in lipid peroxidation caused by CCl_4 exposure in lungs and kidneys of the chicks. These results were in close agreement as reported by researchers earlier[22,30-31]. This study indicated that supplementation of NPME @ 200 mg kg^{-1} resulted in a significant increase in the levels of GSH, POD and CAT. These results suggested the protective role of NPME against CCl_4 induced toxicity. The findings of the current study were in agreement with the result reported by other researchers[30,32].

Chicks intoxicated with CCl_4 and treated with *Nicotiana plumbaginifolia* had a better body weight gain that suggested the protective role of NPME. Decreased body weight and increased weight of kidneys and lungs in chicks fed CCl_4 were comparable with the results reported earlier[33]. The concentration of TBARS in kidney and lung tissues of the control conditions samples was low and was significantly increased in CCl_4 administrated samples. This increase in TBARS was normalized by the supply of NPME in dose-dependent manner[34].

This study is to determine the role of the natural products against the CCl_4 induced toxicity which showed great results. The extract of this plant may not have side effect and it may be used as a medicine for the treatment of stress and anxiety in human beings.

As in this study, the crude extract has been used so further study is required to isolate the bioactive compounds and application of the purified compounds against the stress conditions. Additional to the above, the molecular mechanism of the stress prevention is not yet known which is necessary to determine. Further study is needed in mouse model and ultimately applications of the isolated compounds in human beings.

CONCLUSION

Results of the study showed that NPME contributes its protective role by decreasing the effect of CCl_4 at various metabolic pathways. Based on this study, it may be concluded that NPME may be used in renal and lungs disorders in folk medicines because of the presence of bioactive constituents in this specific medicinal plant.

SIGNIFICANCE STATEMENT

Being byproducts of the normal metabolic processes, reactive oxygen species may cause oxidative stress that ultimately causes different disorders. This oxidative stress may also be induced by carbon tetrachloride (CCl_4) and in the result; free radicals are created in different tissues like lungs, kidneys, heart and blood cells. Antioxidant enzymes prevent the formation of reactive oxygen species as well as scavenge those to rescue the biological systems from the harmful effect of such factors. Here results reported the use of natural antioxidants instead of the synthetic ones against the oxidative stress caused by CCl_4. Crude extract of *Nicotiana plumbaginifolia* has been used as a source of natural antioxidant against CCl_4 intoxication in chicks.

REFERENCES

1. Covarrubias, L., D. Hernandez-Garcia, D. Schnabel, E. Salas-Vidal and S. Castro-Obregon, 2008. Function of reactive oxygen species during animal development: Passive or active?. Dev. Biol., 320: 1-11.
2. Gruber J., S. Schaffer and B. Halliwell, 2008. The mitochondrial free radical theory of ageing - where do we stand? Front. Biosci., 13: 6554-6579.

3. Tsai, J.C., C.S. Chiu, Y.C. Chen, M.S. Lee and X.Y. Hao *et al*., 2017. Hepatoprotective effect of *Coreopsis tinctoria* flowers against carbon tetrachloride-induced liver damage in mice. BMC Compl. Alt. Med., Vol. 17. 10.1186/s12906-017-1604-8.
4. McGregor, D. and M. Lang, 1996. Carbon tetrachloride: Genetic effects and other modes of action. Mutat. Res./Rev. Mutat. Res., 366: 181-195.
5. Lee, T.Y., G.J. Wang, J.H. Chiu and H.C. Lin, 2003. Long-term administration of *Salvia miltiorrhiza* ameliorates carbon tetrachloride-induced hepatic fibrosis in rats. J. Pharm. Pharmacol., 55: 1561-1568.
6. Khan, R.A., M.R. Khan and S. Sahreen, 2010. Evaluation of *Launaea procumbens* use in renal disorders: A rat model. J. Ethnopharmacol., 128: 452-461.
7. Dogukan, A., N. Akpolat, H. Celiker, N. Ilhan, I.H. Bahcecioglu and A.I. Gunal, 2003. Protective effect of interferon-alpha on carbon tetrachloride-induced nephrotoxicity. J. Nephrol., 16: 81-84.
8. Adewole, S., A. Salako, O. Doherty and T. Naicker, 2007. Effect of melatonin on carbon tetrachloride-induced kidney injury in wistar rats. Afr. J. Biomed. Res., 10: 34-41.
9. Weber, L.W., M. Boll and A. Stampfl, 2003. Hepatotoxicity and mechanism of action of haloalkanes: Carbon tetrachloride as a toxicological model. Crit. Rev. Toxicol., 33: 105-136.
10. Brautbar, N. and J. Williams, 2002. Industrial solvents and liver toxicity: Risk assessment, risk factors and mechanisms. Int. J. Hyg. Environ. Health, 205: 479-491.
11. Fruhwirth, G.O. and A. Hermetter, 2008. Mediation of Apoptosis by Oxidized Phospholipids. In: Lipids in Health and Disease, Quinn, P.J. and X. Wang (Eds.)., Springer, Dordrecht, pp: 351-367.
12. Khan, M.R., W. Rizvi, G.N. Khan, R.A. Khan and S. Shaheen, 2009. Carbon tetrachloride-induced nephrotoxicity in rats: Protective role of *Digera muricata*. J. Ethnopharmacol., 122: 91-99.
13. Rajesh, M.G. and M.S. Latha, 2004. Protective activity of *Glycyrrhiza glabra* Linn. on carbon tetrachloride-induced peroxidative damage. Indian J. Pharmacol., 36: 284-287.
14. Mizuguchi, S., S. Takemura, Y. Minamiyama, S. Kodai and T. Tsukioka *et al*., 2006. S-allyl cysteine attenuated CCl_4-induced oxidative stress and pulmonary fibrosis in rats. BioFactors, 26: 81-92.
15. Zhang, H.Q., Y.F. Yau, K.Y. Szeto, W.T. Chan, J. Wong and M. Li, 2007. Therapeutic effect of Chinese medicine formula DSQRL on experimental pulmonary fibrosis. J. Ethnopharmacol., 109: 543-546.
16. Hwang, Y.P., C.Y. Choi, Y.C. Chung, S.S. Jeon and H.G. Jeong, 2007. Protective effects of puerarin on carbon tetrachloride-induced hepatotoxicity. Arch. Pharm. Res., 30: 1309-1317.
17. Fu, Y., S. Zheng, J. Lin, J. Ryerse and A. Chen, 2008. Curcumin protects the rat liver from CCl_4-caused injury and fibrogenesis by attenuating oxidative stress and suppressing inflammation. Mol. Pharmacol., 73: 399-409.
18. Kuleci, S., I. Hanta, A. Kocabas and N. Canacankatan, 2008. The effect of different treatment modalities on oxidative stress in COPD. Adv. Ther., 25: 710-717.
19. Suzuki, S., S. Matsukura, H. Takeuchi, M. Kawaguchi and K. Ieki *et al*., 2008. Increase in reactive oxygen metabolite level in acute exacerbations of asthma. Int. Arch. Allergy Immunol., 146: 67-72.
20. Sackesen, C., H. Ercan, E. Dizdar, O. Soyer and P. Gumus *et al*., 2008. A comprehensive evaluation of the enzymatic and nonenzymatic antioxidant systems in childhood asthma. J. Allergy Clin. Immunol., 122: 78-85.
21. Niki, E., 2010. Assessment of antioxidant capacity *in vitro* and *in vivo*. Free Radical Biol. Med., 49: 503-515.
22. Shah, A.S., M. Ahmed, H.M. Alkreathy, M.R. Khan, R.A. Khan and S. Khan, 2014. Phytochemical screening and protective effects of *Trifolium alexandrinum* (L.) against free radical-induced stress in rats. Food Sci. Nutr., 2: 751-757.
23. Lowry, O.H., N.J. Rosebrough, A.L. Farr and R.J. Randall, 1951. Protein measurement with the folin phenol reagent. J. Biol. Chem., 193: 265-275.
24. Manna, P., M. Sinha and P.C. Sil, 2006. Aqueous extract of *Terminalia arjuna* prevents carbon tetrachloride induced hepatic and renal disorders. BMC Complementary Altern. Med., 6: 33-33.
25. Chance, B. and A.C. Maehly, 1955. Assay of catalases and peroxidases. Methods Enzymol., 2: 764-775.
26. Carlberg, I. and B. Mannervik, 1975. Purification and characterization of the flavoenzyme glutathione reductase from rat liver. J. Biol. Chem., 250: 5475-5480.
27. Mohandas, J., J.J. Marshall, G.G. Duggin, J.S. Horvath and D.J. Tiller, 1984. Differential distribution of glutathione and glutathione-related enzymes in rabbit kidney: Possible implications in analgesic nephropathy. Biochem. Pharmacol., 33: 1801-1807.
28. Habig, W.H., M.J. Pabst and W.B. Jakoby, 1974. Glutathione S-transferases the first enzymatic step in mercapturic acid formation. J. Biol. Chem., 249: 7130-7139.
29. Wright, J.R., H.D. Colby and P.R. Miles, 1981. Cytosolic factors which affect microsomal lipid peroxidation in lung and liver. Arch. Biochem. Biophys., 206: 296-304.
30. Shah, A.S., R.A. Khan, M. Ahmed and N. Muhammad, 2016. Hepatoprotective role of *Nicotiana plumbaginifolia* Linn. against carbon tetrachloride-induced injuries. Toxicol. Ind. Health, 32: 292-298.

31. Khan, R.A., M.R. Khan and S. Sahreen, 2012. Protective effect of *Sonchus asper* extracts against experimentally induced lung injuries in rats: A novel study. Exp. Toxicol. Pathol., 64: 725-731.
32. Ajuwon, O.R. and O.M. Idowu, 2010. Vitamin C attenuates copper-induced oxidative damage in broiler chickens. Afr. J. Biotechnol., 9: 7525-7530.
33. Solcan, C., M. Gogu and G. Solcan, 2011. Protective effect of *Hypophae rhamnoides* oil against ochratoxicosis in chickens. Bull. UASVM Vet. Med., 68: 350-358.
34. Lakshmi, D.U., K. Adilaxmamma, A.G. Reddy and V.V. Rao, 2011. Evaluation of herbal methionine and *Mangifera indica* against lead-induced organ toxicity in broilers. Toxicol. Int., 18: 58-61.