The Possible protective effects of *Physalis peruviana* on carbon tetrachloride-induced nephrotoxicity in male albino rats

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Abstract: *Physalis peruviana* (physalis) has long held a place in folk medicine in the tropical countries where it grows. Here, the aim of this study was to evaluate the potential nephroprotective impact of physalis extract against carbon tetrachloride (CCl_4)-induced kidney injury and to explore the possible mechanisms by which this plant exerts its beneficial effects. Rats were randomly divided into equal four groups, eight rats each. **I. Control Group:** served as Control group. **II. Physalis group**: rats were treated with Physalis extract in drinking water at a dose of 150 mg/kg b.wt. **III. CCl_4 group:** rats were treated with CCl_4 at a dose of 2 mL/Kg b.wt. and **IV. CCl_4 and physalis group**: rats were treated with Physalis extract in drinking water and CCl_4 at the previous doses for 12 weeks. At the end of the experiment, blood samples were collected and used for determination of kidney function, namely; uric acid, urea and creatinine, while the kidney tissues were subjected to hematoxylin and eosin and Bcl-2 immunostaining examination. Evaluation of malondialdehyde (MDA), nitric oxide (NO), glutathione (GSH) were conducted and the activity of superoxide dismutase (SOD), catalase (CAT), glutathione peroxidase (GPx) and glutathione-S-transferase (GST) were carried out. Animals treated with CCl_4 exhibited significant elevations in kidney function, MDA, NO and exhibited significant decrease in the activities of SOD, CAT, GPx, GST and GSH contents. The combination (both physalis and CCl_4) group has preserved the kidney histology, kidney function near to control, exhibited a significant induction in the activities of CAT, SOD and GST, increased the kidney content of GSH and Bcl-2 and conversely showed significant decrease in kidney MDA and NO levels compared to CCl_4-treated rats. Physalis confers an appealing nephroprotective effect which might be explained partially via diminishing the generation of MDA and NO, induction of antioxidant systems and Bcl-2.

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1. Introduction

Oxidative stress results from the imbalance of reactive oxygen species (ROS) and defense mechanisms which results in cell damage. Reactive oxygen species (ROS), including superoxide radicals (O_2•-), hydrogen peroxide (H_2O_2) and hydroxyl radicals (OH•) are generated as byproducts of normal metabolism (Rice-Evans and Miller, 1996). Cumulative oxidative damage leads to numerous diseases and disorders (Halliwell, 1991). The enhanced production of free radicals and oxidative stress can also be induced by a variety of factors such as radiation or exposure to heavy metals and xenobiotics e.g. carbon tetrachloride (Kim *et al.,* 1990).

Carbon tetrachloride (CCl_4) is an industrial solvent with a strong nephrotoxin, extensively used to induce oxidative stress in laboratory animals. CCl_4 toxicity results from its bioactivation to trichloromethyl free radical by cytochrome P450 isozymes (Raucy *et al.,* 1993). The trichloromethyl radical reacts with oxygen to form the highly toxic reactive trichloromethyl peroxy radical, a reactive oxygen species (ROS). Free radical-induced lipid peroxidation believed to be one of the major causes of cell membrane damage, depletion of antioxidant status and DNA injuries in kidneys of rat leading to a number of pathological situations (Khan *et al.,* 2010). Reports from Ogeturk *et al.*(2005b) suggested that acute and chronic renal injuries occur due to the exposure to carbon tetrachloride. Kidney tissue has great affinity for CCl_4 because of the predominant presence of the cytochrome p450 in the cortex. Previous reports suggest that CCl_4 generates free radicals with the implication of pathological environment by damaging the integrity of cell membranes, elevating thiobarbituric acid reactive substances (TBARS) level with subsequent necrosis and affecting physical parameters of kidney such as urinary and serum profile (Sahreen *et al.,* 2011). Renal sources for ROS are activated macrophages, vascular cells, and various glomerular cells. ROS may affect cells of the host organism, especially at sites of inflammation in addition to playing a role in the defense system against other agents. This effect plays a role in a variety of renal diseases such as glomerulonephritis and tubulointerstitial nephritis which can contribute to

proteinuria and other conditions (Ichikawa *et al.,* 1994). The presence of inflammation is well-documented factor influencing the development of oxidative stress in dialysis patients (Samouilidou *et al.,* 2003).

It has been noticed that many of plants which are rich in phenolic compounds, are widely used as antioxidant and antimutagenic (Sivalokanathan *et al.,* 2006). High consumption of fruits and vegetables is associated with low risk for several degenerative diseases, such as coronary artery disease, stroke, rheumatoid arthritis, diabetes and cancer, which is attributed to the antioxidant vitamins and other phytochemicals (Prior , 2003).

Physalis peruviana Linn. (Solanaceae) is an erect branching densely villous perennials, which are grown for their fruits and for decoration. They grow wild in Europe, America and Asia. It has been used as a folk medicine with antiinflammatory, antitussive, antipyretic, diuretic, antidotal and antitumor effects in Taiwan (Lee *et al.,* 2008). Various chemical compounds like 28-hydroxywithanolide, withanolides, phygrine, kaempferol, and quercetin di- and tri-glycosides are reported to be present in *Physalis peruviana* (Arun and Asha, 2007). Some of these components were found to have antitumor, cytotoxic inhibition of the ubiquitin-proteasome pathway(Ausseil *et al.,*2007), immunomodulatory (Soares *et al.,* 2006), antimycobacterial (Pietro *et al.,* 2000), antiinflammatory and antiallergic activities (Lee *et al.,* 2008).

Physalis peruviana contains biologically active components e.g. physalins, withanolides, phytosterols and polyunsaturated fatty acids e.g. linoleic acid and oleic acid. Among its major components are high amounts of vitamins A,B and C as well as the presence of essential minerals, magnesium, calcium, potassium, sodium and phosphorus which are classified as macronutrients, while the Iron and Zinc are considered as micronutrients (Szefer & Nriagu, 2007).

The fatty acids composition and high amounts of polyunsaturated fatty acids found in oils extracted from physalis peruviana L. make this fruit ideal for nutrition (Ramadan and Morsel, 2003).

The bioactive phytosterols would give them properties such as antioxidant and hypocholesterolemic effects. Furthermore, the antioxidant activity is due to the high levels of polyphenols and high levels of vitamins A and C. Finally, the presence of exclusive Physalis-gender Physalins and withanolides specific from the Solanaceae family would give the fruit of physalis peruviana L. anti-inflammatory, antimicrobial and anticancer properties.

The withanolides are steroidal lactones mainly produced by Solanaceous plants. Its components have antimicrobial properties, antitumor, anti-inflammatory, hepatoprotective or inmunomodulatory and antiparasitic activity (Ahmad *et al.,* 1999; Lan *et al.,* 2009).

The specific active constituents of physalis peruviana L. are Physalins A, B, D, F and glycosides, which show anticancer activity (Wu *et al.,* 2004). Physalins are immunosuppressive substances which are widely used to inhibit unwanted immune responses in autoimmune diseases, allergies and organ transplants.

The fruit of physalis peruviana L. is highly nutritious, having high levels of vitamins A, B and C. The main active components of vitamin A in fruits are α-carotene, β-carotene and β cryptoxanthin (Fischer *et al.,* 2000).

Phytosterols are of great interest because of its antioxidant capacity and impact on both total cholesterol and LDL cholesterol (Ramadan and Morsel, 2003; Valenzuela & Ronco, 2004).

The present study aims to investigate the possible protective effect of *Physalis peruviana* extract against carbon tetrachloride-induced nephrotoxicity and apoptosis.

2. Materials and Methods

2.1. Animals

Adult male Wistar albino rats weighing 120–150g (7-9 weeks old) were obtained from The Holding Company for Biological Products and Vaccines (VACSERA, Cairo, Egypt). Animals were kept in wire bottomed cages in a room under standard condition of illumination with a 12-hours light-dark cycle, 55+5% relative humidity and at 25±2ºC room temperature. They were provided with balanced standard pellet (VACSERA) as a diet and tap water *ad libitum*. The animals were kept in a clean and low stress environment with an enclosed door. The experiments were approved by the state authorities and followed Egyptian rules on animal protection.

2.2. Physalis peruviana extract preparation

Fresh fruits of physalis were purchased from local market in Cairo. The samples were identified in Botany Department, Faculty of Science, Helwan University. The fruits were cleaned, dried and minced, the juice and residue were used for the preparation of crude methanolic extract by percolation at room temperature with 70% methanol alcohol and kept in refrigerator for 24 hours. Extract of physalis was concentrated under reduced pressure (bath temperature 50 ºC) and dried in a vacuum evaporator. The residue was dissolved in distilled water, filtered and used in experiments.

2.3. Measurement of flavonoids, total polyphenols and *in vitro* free radical scavenging assays

For the assessment of flavonoids, colorimetric method introduced by Dewanto *et al.* (2002) was adapted. To determine the amount of flavonoids by the above mentioned method, 1.50 ml of the deionized water was added to 0.25 ml of the sample and then 90 µl of 5% Sodium nitrite ($NaNO_2$).Six min later, after

addition of 180 µl of 10% $AlCl_3$, mixture was allowed to stand for another 5 min before mixing 0.6 ml of 1M NaOH. By adding deionized water and mixing well, final volume was made up to 3 ml. Using blank, absorbance was measured at 510 nm. Calibration curve was prepared using quercetin acid as standard for total flavonoids which was measured as mg quercetin equivalents (QE) per milliliter of the sample (µg/ ml).

The total polyphenolic contents (TOC) were measured using Folin-Ciocalteau reagent by the method of Kim *et al.* (2003) based on the oxidation of polyphenols to a blue colored complex with an absorbance maximum of 700 nm. Calibration curve was prepared using gallic acid as standard for TPC which was measured as mg gallic acid equivalents (GAE) per milliliter of the sample (µg/ ml).

The free radical scavenging capacity was evaluated by 2, 2-Diphenyl -1- picrylhydrazyl (DPPH) assay described by (Burits and Bucar, 2000). In its radical form, DPPH absorbs at 517 nm, but upon reduction by an antioxidant or a radical species, the absorption decreases. Briefly, 1ml of 0.25 mM solution of DPPH in methanol was added to 50, 100, 150 and 200 ul of sample in 950, 900, 850 and 800 µl methanol, respectively. After 20 min, the absorbance was measured at 517 nm. Ascorbic acid was used as a reference standard. The percentage DPPH decolorisation of the sample was calculated by the equation: % DPPH scavenging=$[(A_{control}-A_{sample})/A_{control}]$-100, where A is the absorbance.

The total antioxidant potential was measured by the ability of the sample to scavenge thiobarbituric acid-reactive substances (TBARS) (Tripathi and Sharma, 1998). Briefly, 50, 100, 150 and 200 ul of the different samples were added to the 10% liver homogenate. Lipid peroxidation was initiated by addition of 100 µl of 15 mM $FeSO_4$ solution to 3 ml of liver homogenate (final concentration was 0.5 mM). After 30 min, 100 µl of this reaction mixture was taken in a tube containing 1.5 ml of 0.67% thiobarbituric acid (TBA) in 50% acetic acid. Samples were incubated at 37°C for 1 hr, and then lipid peroxidation was measured using the reaction with TBA. The absorbance of the organic layer was measured at 532 nm. All reactions were carried out in triplicates. Vitamin C was used as a reference standard. The percentage of inhibition of lipid peroxidation was calculated, by the formula: Inhibition (%)=$(A_{control}-A_{sample})X100/A_{control}$.

The superoxide anion scavenging activity was determined by the method of Nishimiki *et al.* (1972). Superoxide anion derived from dissolved oxygen by a phenazine methosulfate (PMS)/NADH coupling reaction reduces nitroblue tetrazolium (NBT), which forms a violet colored complex. A decrease in color after addition of the antioxidant is a measure of its superoxide scavenging activity. To the reaction mixture containing phosphate buffer (100 mM, pH 7.4), NBT (1 mM) solution, NADH (1 mM) and 50, 100, 150 and 200 ul of sample in 950, 900, 850 and 800 µl methanol, respectively, 1 ml of 1 mM PMS was added. After incubation at 25 °C for 5 min, the absorbance was measured at 560 nm against a blank. Vit. C was used as a reference standard.

The nitric oxide radical inhibition activity was measured by the method of Garratt (1964) using Griess reagent. Briefly, sodium nitroprusside (10 mM) in phosphate buffered saline was mixed with 50, 100, 150 and 200 ul of sample in 950, 900, 850 and 800 µl methanol, respectively, and incubated at room temperature for 150 min followed by addition of 0.5 ml of Griess reagent (1% sulfanilamide, 2% H_3PO_4 and 0.1% N-(1-naphthyl)ethylenediamine dihydrochloride). The absorbance of the chromophore formed was read at 546 nm.

2.4 Experimental protocol

Animals after acclimatization (7 days) in the animal quarters were fasted overnight and randomly divided into 4 groups of 8 animals each and treated in the following way:

Group I served as Control.

Group II Rats received physalis extract 150 mg/kg daily in drinking water orally for 12 weeks.

Group III Rats were injected with carbon tetrachloride (2mL/Kg, i.p.) once weekly for 12 weeks (Sohn *et al.,* 1991).

Group IV Rats received physalis extract150 mg/kg daily in drinking water for 12 weeks, also, the rats were injected with carbon tetrachloride (2mL/Kg b.wt., i.p.) once weekly for 12 weeks .

Animals were killed after 24 hrs from the last dose. Blood was collected by cervical dislocation and allowed to clot for 30 min at room temperature. The serum was separated by centrifugation at 3000 rpm at 4 °C for 15 min and used for the estimation of the marker kidney function parameters namely, uric acid, urea and creatinine. The kidney were dissected out immediately, washed with ice cold saline and 10% homogenates in 50 mM Tris-HCl and 300 mM sucrose were prepared. The homogenates were centrifuged at 5000 ×g for 10 min at 4 °C and the supernatants were used for the assay of MDA, NO, GSH, SOD, CAT, GPx, GST and GR.

2.5 Kidney index

At the end of the experimental period, each rat was weighted. The left kidney was then removed and weighed. Finally, the kidney index was calculated by dividing left kidney weight by body weight and then multiplying it by 100.

2.6 Biochemical estimations

2.6.1 Kidney function tests

Serum uric acid (sUA), urea (sU) and serum creatinine (sCr) were assayed using kits provided from Biodiagnostic Co. (Giza, Egypt) according to the

methods described by Fossati *et al.* (1980), Fawcett and Soctt (1960) and Szasz *et al.* (1979) respectively.

2.6.2 Malondialdehyde (MDA) in kidney

Levels of MDA were assayed by the method of Satoh (1978). Briefly, 0.2mL supernatant of kidney homogenate was mixed with 0.67% 2- thiobarbituric acid (TBA) and 20% trichloroacetic acid solution, and heated in a boiling water bath for 30 min. The pink-colored chromogen formed by the reaction of TBA with MDA was measured at 532 nm. The results were expressed as MDA nmol/g tissue.

2.6.3 Nitric oxide (NO) in kidney

Nitric oxide level in kidney tissue homogenates was determined according to the method of Ignarro *et al.* (1987). The assay is based on the diazotization of sulfanilic acid with nitric oxide at acidic pH and subsequent coupling with N-(10-naphthyl)-ethylenediamine to yield an intensely pink colored product that is measured spectrophotometrically at 540 nm.

2.6.4 Glutathione (GSH) in kidney

Levels of GSH in kidney homogenates were assayed by the method of Beutler *et al.* (1963). Briefly, the deproteinzation of kidney homogenate was made by 10% trichloroacetic acid and centrifuged at 3500 rpm for 10 min. 50µl supernatant was mixed with 0.32 mol/l disodium hydrogen phosphate and 0.04% 5,5'-dithiobis 2-nitrobenzoic acid (DTNB) solution. The yellow-colored substance formed by the reaction of GSH and DTNB was measured at 412 nm. The results were expressed as GSH mg/g tissue.

2.6.5 Antioxidant enzymes in kidney

a. Catalase (CAT) was determined spectrophotometrically by following the decomposition of H_2O_2 in 50 mM potassium phosphate buffer, pH 7.0, at 240 nm as described by Aebi (1984).
b. Superoxide dismutase (SOD) activity in kidney homogenate was determined according to the method of Minami and Yoshikawa (1979). This method is based on the generation of superoxide anions by pyrogallol autoxidation, detection of generated superoxide anions by nitro blue tetrazolium (NBT) formazan color development and measurement of the amount of generated superoxide anions scavenged by SOD (the inhibitory level of formazan color development).
c. Glutathione-S-transferase (GST) activity in kidney homogenate was determined by the method described by Habig *et al.* (1974). The conjugation of 1- chloro- 2,4- dinitrobenzene (CDNB) with reduced glutathione was measured. The conjugation is accompanied by an increase in absorbance at 340 nm where the rate of increase in the absorbance was directly proportional to the GST activity in the homogenate.
d. Glutathione reductase activity was determined spectrophotometrically as described by Bilzer *et al.* (1984). GR catalyses the reduction of glutathione (GSSG) in the presence of reduced nicotinamide adenine dinucletide phosphate (NADPH), which is oxidized, to $NADPH^+$. The decrease in absorbance at 340 nm was measured.
e. Glutathione peroxidase (GPx) was determined in kidney homogenate according to the method of Lawrence and Burk (1976). This method is based on measuring the oxidation of NADPH using hydrogen peroxide as the substrate. Absorbance was measured at 340 nm for 5 minutes, and an extinction coefficient of 6.22×10^{-3} was used for calculation. The results were expressed as µmol/min/gm tissue. The changes in the absorbance at 340 nm were recorded at 1-min interval for 5 min.

2.6.6 Histological assessment

Kidneys from rats of different groups were fixed in 10% neutral formalin solution, dehydrated in graded alcohol and embedded in paraffin. Fine sections obtained were mounted on glass slides and counter-stained with hematoxylin–eosin (H&E) for light microscopic analysis (Culling, 1974).

2.6.7 Immunohistochemical analyses of Bcl-2

For immunohistochemistry, kidney section (4 µm) were deparaffined and then boiled in Declere (Cell Marque, Hot Springs, AR, USA) to unmask antigen sites; the endogenous activity of peroxidase was quenched with 0.03% H_2O_2 in absolute methanol. Kidney sections were incubated overnight at 4 °C with a 1:200 dilution of anti Bcl-2 antibodies in phosphate buffered saline (PBS). Following removal of the primary antibodies and repetitive rinsing with PBS, slides were incubated with a 1:500 dilution of biotinylated goat anti-IgG secondary antibody. Bound antibodies were detected with avidin biotinylated peroxidase complex ABC-kit Vectastain and diaminobenzidine substrate. After appropriate washing in PBS, slides were counterstained with hematoxylin. All sections were incubated under the same conditions with the same concentration of antibodies and at the same time; so the immunostaining was comparable among the different experimental groups (Bancroft & Cook, 1994).

2.6.8 Statistical analysis

The obtained data were presented as means ± standard error. One-way ANOVA was carried out, and the statistical comparisons among the groups were performed with Duncan's test using a statistical package program (SPSS version 17.0). All *P*-values are two-tailed and $P<0.05$ was considered significant for all statistical analysis in this study.

3. Results

Physalis extract has shown positive tests for gallic tannins, while the extract has given negative result for catechol tannins (Table 1).

Table 1: Quantitative analysis of tannins and its type in physalis extract.

Parameter	Amount
Gallic Tannins	+
Catechol Tannins	-

Figure 1(A) has shown the flavonoids and total polyphenolic contents of physalis extract. Flavonoids content in physalis extract was 89.4 µg/ mg quercetin equivalents of flavonoids. The total polyphenolic content was 121.3 µg/ mg gallic acid equivalent of polyphenols.

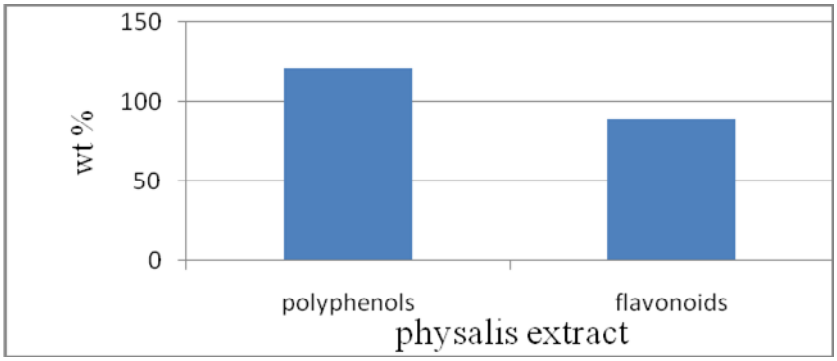

Figure 1(A): Total flavonoids and polyphenolic contents of physalis extract. (a) Flavonoids are expressed as µg/ mg quercetin equivalents of flavonoids. (b) Total polyphenols are expressed as µg/ mg gallic acid equivalent of polyphenols. Data are represented as mean ± SEM of two independent experiments each performed in duplicate.

Free Radical Scavenging Activity

As shown in Fig. 1 (B), Physalis extract revealed a concentration-dependent free radical scavenging activities resulting from reduction of DPPH and superoxide radicals. Physalis extract at concentrations of 20-100 µg/ml, have shown that it possesses a scavenging activities ranging from 10-100%. The scavenging activity of Vit. C, a known antioxidant,was used as positive control.

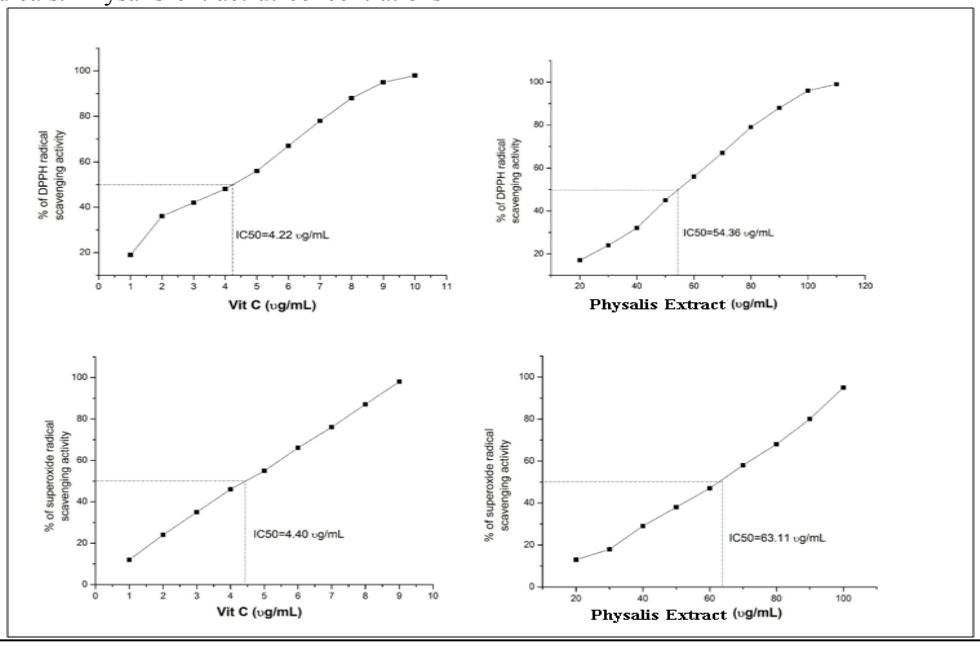

Fig. 1 (B) presents the Inhibition of DPPH and superoxide radicals by physalis extract. Data are represented as mean ± SEM of two independent experiments each performed in duplicate.

As shown in Fig. 1 (C), Physalis extract revealed a concentration-dependent free radical scavenging activities resulting from reduction of TBARS and nitric oxide radicals. Physalis extract at concentrations of 20-100 µg/ml, have shown that it possesses a scavenging activities ranging from 10-100%. The scavenging activity of Vit. C, a known antioxidant,was used as positive control.

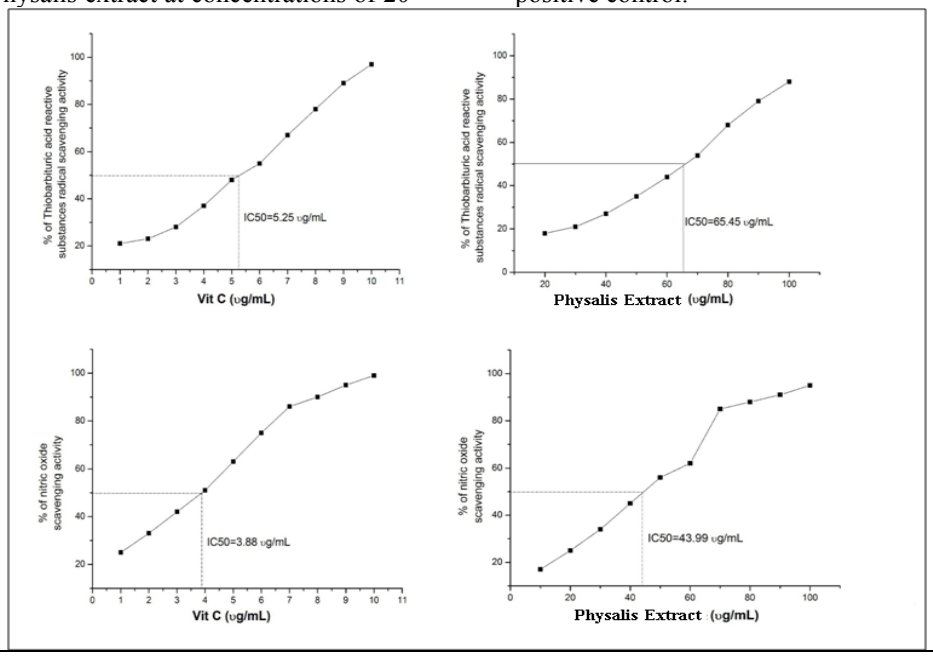

Fig. 1 (C) presents the Inhibition of TBARS and nitric oxide radicals by physalis extract. Data are represented as mean ± SEM of two independent experiments each performed in duplicate

Effect of physalis extract and CCl_4 on kidney relative weight

As shown in (Fig. 2) carbon tetrachloride induced a significant increases in kidney weight and relative kidney weight of rats due to a significant decrease in body weight and kidney swelling . Physalis extract markedly reduced this swelling but the kidney weight was still significant increased when compared with the control group.

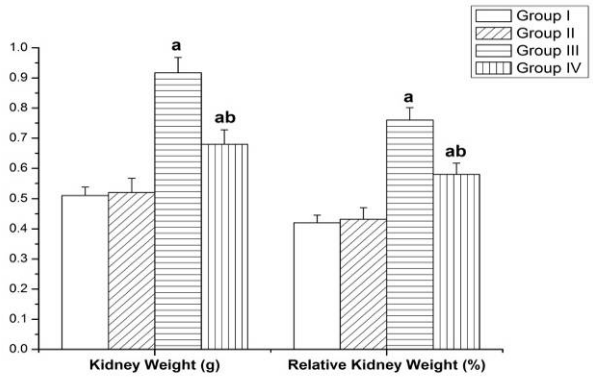

Fig. 2: Effects of physalis extract on kidney weight and relative kidney weight in rats treated with CCl_4. **a:** Significantly different from control group. **b**: Significantly different from CCl_4 group. ANOVA followed by Duncan's test at $p < 0.05$.

Effect of physalis extract and CCl_4 on kidney function tests

The present study showed that administration of CCl_4 to rats caused a significant increase in creatinine (48%), urea (19.8%) and uric acid (49.51%) compared with control group (Table 2), moreover, concomitant administration of physalis extract and CCl_4 to rats caused a significant decrease by 34.26% in uric acid,20.6% in urea and 37.83% in creatinine levels compared to CCl_4 group. Administration of physalis extract alone caused significant decrease in serum creatinine level by 22% versus the control group.

Table (2) Effect of physalis extract on carbon tetrachloride-induced elevations of kidney function parameters of male albino rats.

Groups	sU (mg/dl)	sUA (mg/dl)	sCr (mg/%)
Group I	69.63±4.01	4.10±0.19	0.50±0.01
Group II	58.01±1.58	4.32±0.06	0.39±0.02[a]
Group III	83.42±3.97[a]	6.13±0.02[a]	0.74±0.03[a]
Group IV	65.82±1.85[b]	4.03±0.16[b]	0.46±0.02[b]

Data are expressed as means ± SEM of eight rats. **a:** Significantly different from control group at $p < 0.05$.
b: Significantly different from CCl_4 group at $p < 0.05$.

Effect of physalis extract and CCl_4 on MDA and NO concentrations

The effect of physalis extract on CCl_4-induced elevations of lipid peroxidation (expressed as MDA) and nitric oxide levels in the kidney tissue homogenate are shown in (Fig. 3). CCl_4 significantly increased the renal MDA and NO levels compared with control group. Concomitant treatment with Physalis extract and CCl_4 markedly decreased MDA and NO levels compared with CCl_4 group. Moreover, treatment with physalis extract alone caused a significant decrease in MDA and nitric oxide concentrations in kidney tissue homogenate compared with control group.

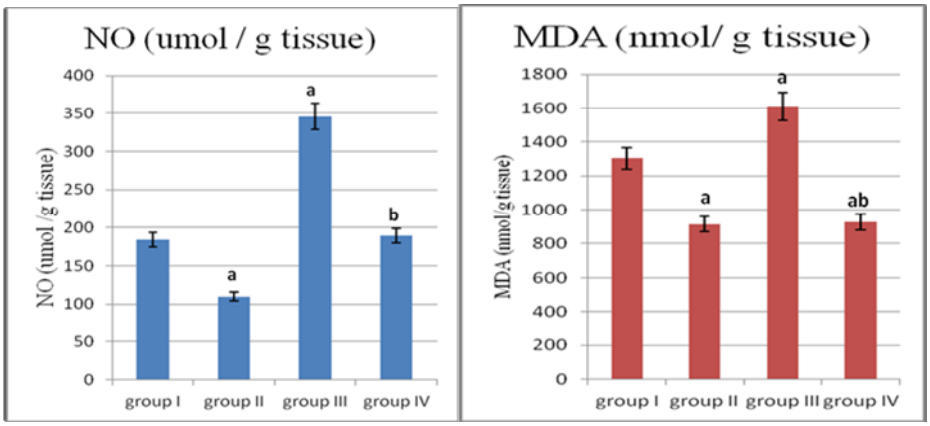

Fig. 3: Protective role of physalis extract on renal MDA and NO levels in rats treated with CCl_4. **a:** Significantly different from control group. **b:** Significantly different from CCl_4 group. ANOVA followed by Duncan's test at $p < 0.05$.

Effect of physalis extract and CCl_4 on reduced glutathione levels

Treatment with physalis caused a significant increase in GSH levels in the kidney homogenate, while treatment with CCl_4 induced a significant decrease in GSH levels compared to the control group. Moreover, when rats were treated along with physalis extract and CCl_4 there was a significant elevation in GSH levels in kidney tissue homogenate compared to CCl_4 group as shown in (Fig.4).

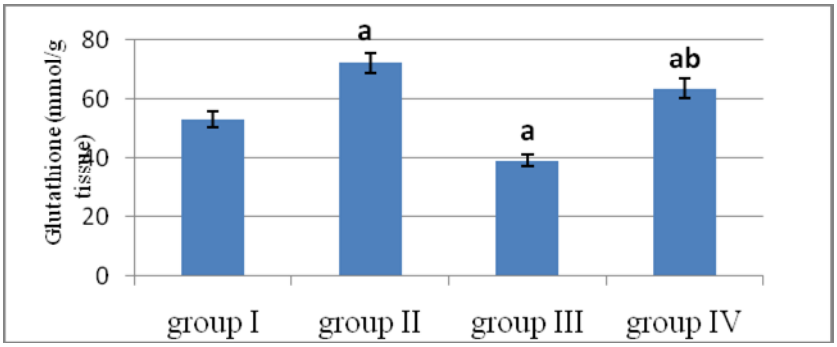

Fig. 4: Protective role of physalis extract on renal GSH level in rats treated with CCl_4. **a:** Significantly different from control group. **b:**Significantly different from CCl_4 group. ANOVA followed by Duncan's test at $p < 0.0$

Effect of physalis extract and CCl_4 on the activities of the antioxidant enzymes

AS shown in Table (3), the activities of antioxidant enzymes were markedly decreased in kidney tissue homogenates of rats treated with CCl_4 as follow: GPx (45.14%), GST (44.91%) and GR (49.18%) compared with control group. However, concomitant administration of physalis extract and CCl_4 significantly attenuated the toxic effect of CCl_4 on the activities of the enzymes GPx, GST and GR by 85.29%, 44.17% and 113.37% respectively compared with CCl_4 group. Treatment with physalis extract significantly increased the activities of GR by 19.9% and GPx by 200% compared with the control group.

Table (3) Effect of physalis extract on carbon tetrachloride-induced changes of antioxidant enzymes of kidney of male albino rats

Groups	GR *(µmol/g)*	GST *(µmol/h/g)*	GPx *(U/g)*
Group I	158.75±3.07	0.5137±0.041	1125.77±83.03
Group II	190.34±6.74[a]	0.522±0.004	3377.32±102.33[a]
Group III	80.68±7.41[a]	0.283±0.021[a]	617.57±39.86[a]
Group IV	172.15±10.5[b]	0.408±0.027[ab]	1144.32±66.07[b]

Data are expressed as means ± SEM of eight rats. **a:** Significantly different from control group at $p < 0.05$. **b:** Significantly different from CCl_4 group at $p < 0.05$

Effect of physalis extract and CCl_4 on SOD and CAT activities

Treatment with CCl_4 markedly decreased the activities of SOD and CAT compared with the control group, while concomitant treatment with physalis extract and CCl_4 significantly increased the activity of SOD as compared with CCl_4 group. Treatment with physalis extract and CCl_4 resulted in significant increase in the activity of CAT as compared with CCl_4 group but still significantly decreased compared with the control group as shown in (Fig. 5).

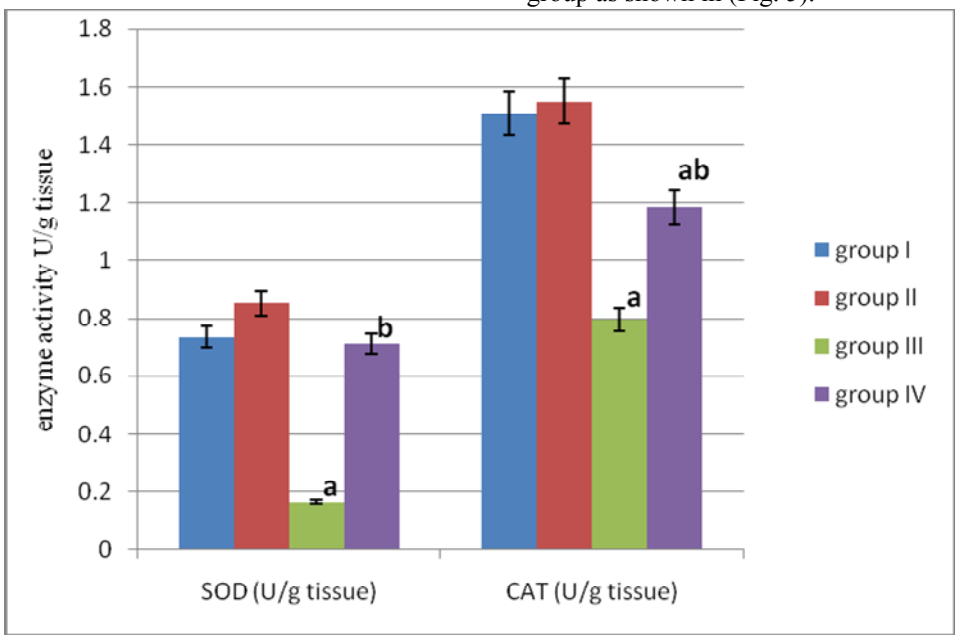

Fig. 5: Protective role of physalis extract on renal SOD and CAT activities in rats treated with CCl_4. **a:** Significantly different from control group. **b:** Significantly different from CCl_4 group. ANOVA followed by Duncan's test at $p < 0.05$.

Effect of physalis extract and CCl_4 on histopathological changes in the kidney

There was no abnormal appearance or histological changes in the kidney of the control rats or in physalis extract treated rats, where there are normal proximal and distal tubules and intact glomerular toufts (Figs. 6A & 6B). CCl_4 injection caused classical damage in the rat kidney after 12 weeks, as demonstrated by congested and swollen glomeruli, decrease in the height of epithelial cells lining convoluted tubules with vaculted cytoplasm and pyknotic nuclei, shedding of atypical cytoplasm and loss of brush boarder (Fig. 6C). Lumen wide opens and marked congestion in vessels were also seen.

Treatment with physalis extract markedly prevented congestion in glomeruli and vessels and other alterations (Fig. 6D).

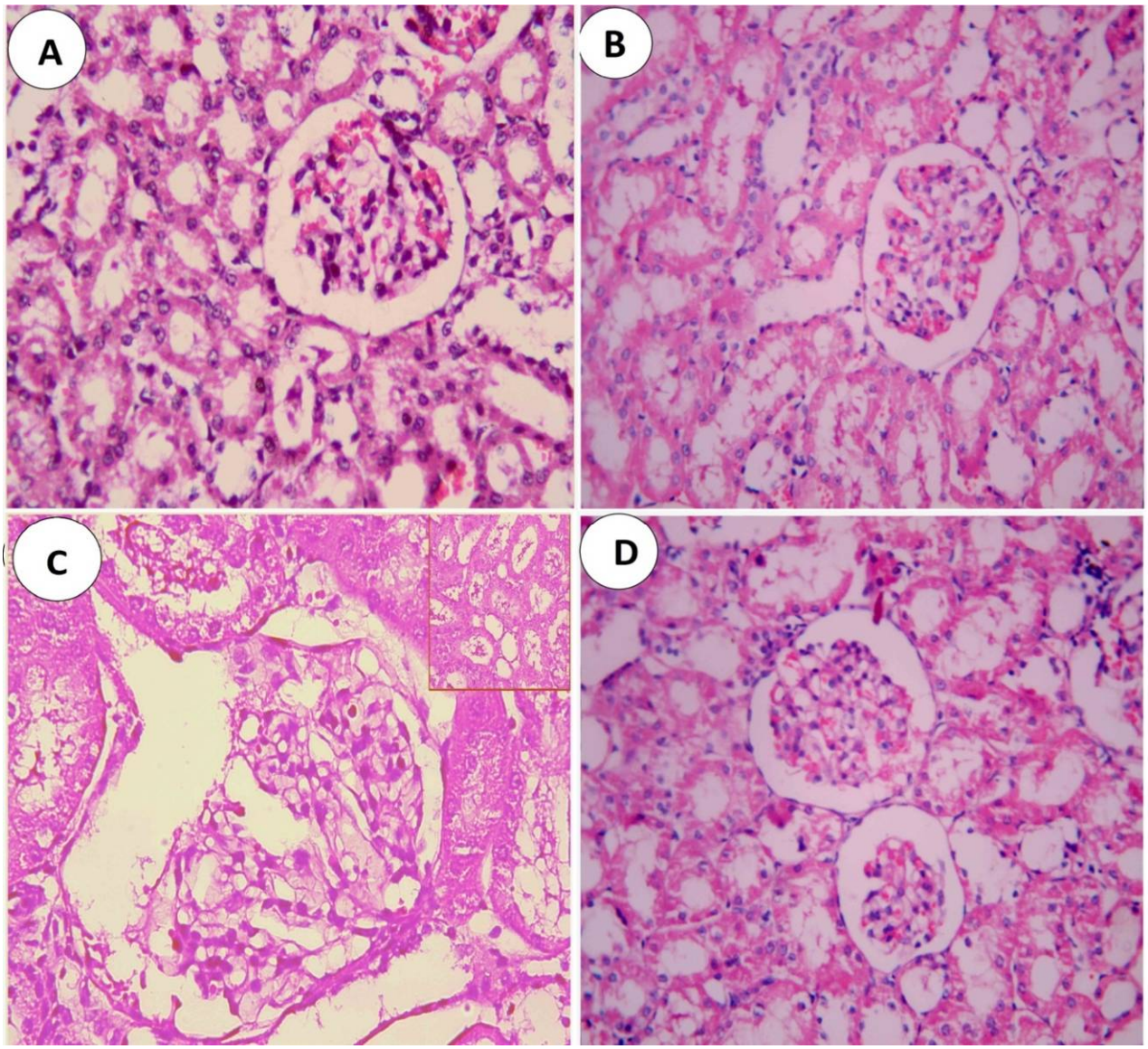

Fig. 6: Effect of physalis extract on histopathological damages induced by CCl_4 on the kidney of rats. Kidney sections were stained using the hematoxylin–eosin method. (A) Control,(B) Physalis extract.(C) CCl_4 and (D) Physalis extract and CCl_4. Original magnifications 400×.

Effect of physalis extract and CCl_4 on immunostaining activity for Bcl-2

Immunohistochemical investigation for Bcl-2 showed that there was some immuno reactivity on the kidney indicating the normal life cycle of cells (Fig. 7). The immunostaining activity for Bcl-2 was decreased in CCl_4 group indicating the apoptotic effect of CCl_4. The protective effect of physalis extract was shown when rats treated with the extract where the numbers of Bcl-2 immunostaining cells were increased indicating the anti-apoptotic effect of physalis extract.

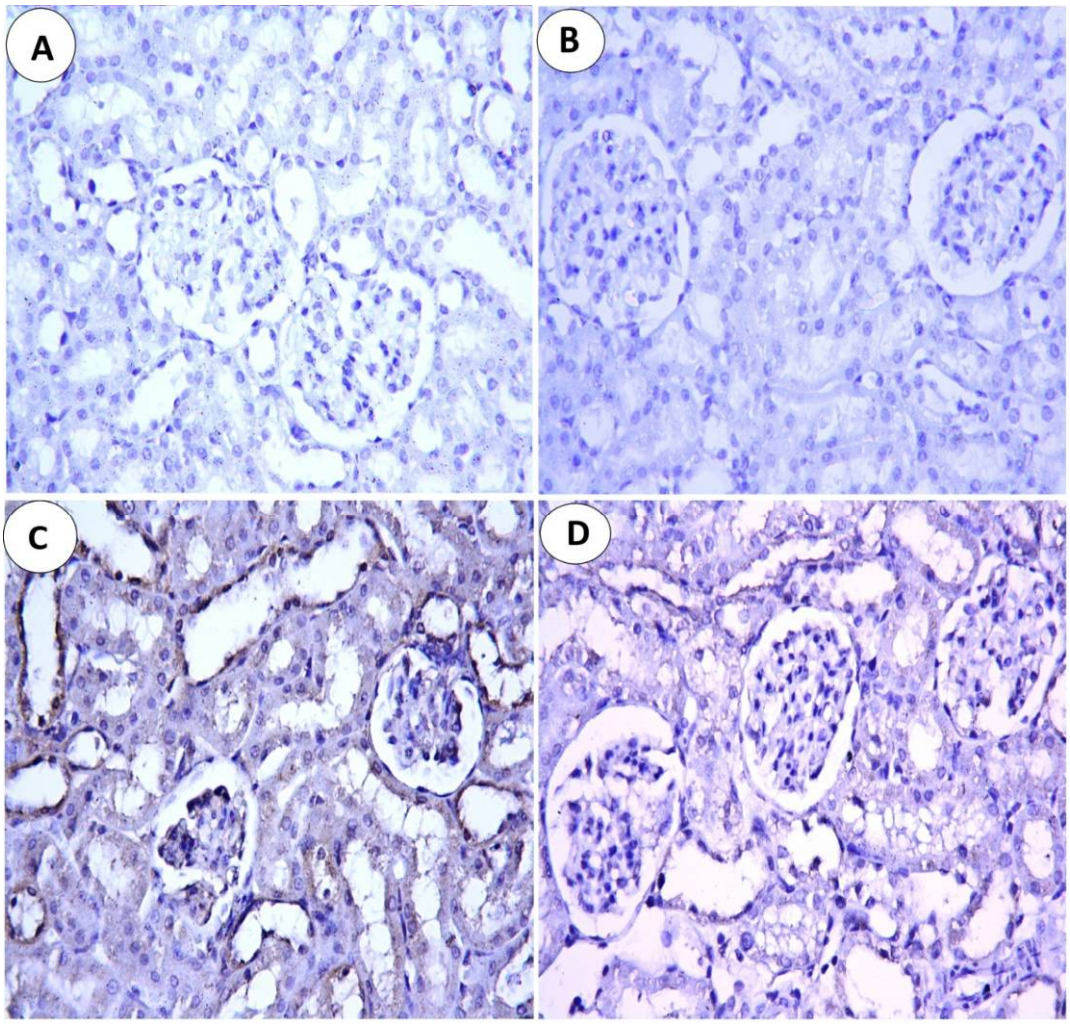

Figure 7: Immunohistochemical localization of Bcl-2 antigen in the kidney tissue of rats. (A) Control, (B) Physalis extract. (C) CCl_4 and (D) Physalis extract and CCl_4. Original magnifications 400×.

4. Discussion

There are various chemicals (xenobiotics) and drugs which cause damage to renal tissues by reactive oxygen species (ROS) production. CCl_4 is known to induce ROS, deplete antioxidant defenses; enzymatic and non-enzymatic substrates; to cause oxidative stress in renal tissues. It was reported that CCl_4 metabolized by cytochrome p- 450 generates a highly reactive free radical, and initiates lipid peroxidation of the cell membrane of the endoplasmic reticulum and causes a chain reaction. These reactive oxygen species can cause oxidative damage in DNA, proteins and lipids (Melin *et al.*, 2000). Various studies have demonstrated that CCl_4 causes free radical generation in many tissues including kidney. Olagunjua *et al.* (2009) suggested a role for reactive oxygen metabolites as one of the postulated mechanisms in the pathogenesis of CCl_4 nephrotoxicity.

It has also been reported that systemically administered CCl_4 in rats was distributed at higher concentrations in the kidney than in the liver (Sanzgiri *et al.,* 1997). Since the kidney has high affinity for CCl_4 (Abraham *et al.,* 1999) and contains cytochrome P450 predominantly in the cortex (Rush *et al.,* 1984; Ronis *et al.,* 1998), CCl_4 is extensively metabolized in the kidney generating more reactive metabolites.

The increment in lipid peroxidation as assessed by the elevated levels of MDA following CCl_4 administration has been well documented in kidneys (Khan *et al.,* 2009). This may be the consequence of an increment in the formation of oxygen free radicals (generated by CCl_4) since antioxidant defense systems are compromised (Priscilla and Prince, 2009).

It has been hypothesized that physalis extract affords protection by impairing CCl_4 mediated lipid peroxidation, through decreased production of free radical derivatives. The antioxidant effect of flavonoids

that was found in physalis enhanced the process of regeneration. This might be due to destruction of free radicals, supplying a competitive substrate for unsaturated lipids in the membrane and/or accelerating the repair mechanism of damaged cell membrane.

The present study has shown that NO level significantly increased in CCl4 treated animals. It has been reported that elevated levels of lipid peroxidation stimulates host cells, mainly monocytes/ macrophages, to produce and release NO by induction of inducible nitric oxide synthase (iNOS) protein, resulting in cytotoxicity and DNA damage (Raso *et al.,* 2001).

The NO radicals play an important role in inducing inflammatory response and their toxicity multiplies only when they react with superoxide anion ($O_2^{\bullet-}$) radicals to form peroxynitrite that damages biomolecules such as proteins, lipids, and nucleic acids (Gulcin *et al.,* 2002; Gouthamchandra *et al.,* 2010).

It was found that nitrite contents in renal tissues were increased in CCl_4-treated rats (Khan *et al.,* 2009).

Nitrites can be converted into nitric oxide (NO) in acidic pH. Peroxynitrite anions have been generated by the reaction of nitric oxide and superoxide anions. These peroxynitrite anions oxidize biomolecules, which finally leads to lipid peroxidation and tubular damage (Muriel, 1998).

In the view of the current data, physalis extract has been found to decrease NO level in rats injected with CCl4. The inhibitory effect of physalis extract on NO may be due to the inhibition of the induction of iNOS protein/enzyme.

The results obtained in this study suggest the protective effects of *Physalis peruviana* extract against CCl_4-induced oxidative stress, could be attributed to its high levels of polyphenols and other antioxidants like flavonoids. These compounds could scavenge the free radicals of CCl_4 generated through cytochrome P450 enzyme system thereby diminished the oxidative injuries.

With regard to the antioxidant defense system in this study, CCl_4 treatment significantly reduced the total contents of GSH, the activity levels of SOD, CAT, GPx, GST and GR indicating that CCl_4 has caused severe oxidative stress. It has been reported that SOD, CAT, GPx, GR and GST constitute a mutually supportive team of defense against ROS (Ji *et al.,* 1988). The decrease of non-enzymatic antioxidant defense is in agreement with the previous findings obtained by Khan *et al.* (2009).

GSH is involved in several defense processes against oxidative damage protects cells against free radicals, peroxides and other toxic compounds (Sies, 1999).

Indeed, glutathione depletion increases the sensitivity of cells to various aggressions and also has several metabolic effects. It is widely known that a deficiency of GSH within living organisms can lead to tissue disorder an injury (Limon-Pacheco *et al.,* 2007).

GSH is used to evaluate the non-enzymatic antioxidant capacity of a tissue to prevent the damage associated to free radical production (Halliwell and Gutteridge, 2007).

Reductive dehalogenation of CCl_4 by the cytochrome P450 enzymes system to the highly reactive trichloromethyl (CCl_3) radical initiates the process of lipid peroxidation which is considered to be the most important mechanism in the pathogenesis of renal damage induced by CCl_4 (Khan *et al.,* 2009). We cannot however exclude the possibility that CCl_4 metabolites may directly react with GSH, reducing its concentration. It was previously observed that CCl_3 radical can even react with sulfhydryl groups of glutathione and protein thiols to alter the redox status of cells (Sheweita *et al.,* 2001). Since GSH is considered an important defense against lipid oxidative damage in the kidneys eliminating hydrogen peroxide, peroxyl and hydroxyl radicals formed during this process, therefore, GSH dependent enzymes will be affected when its level is depleted in the cells (Khan and Ahmed, 2009). It has been suggested that a decrease in the activities of primary antioxidant; SOD and CAT may be due to accumulation of reactive oxygen species. The observation that strengthens this hypothesis is that SOD activity can be inhibited by hydrogen peroxide treatment (Miguel *et al.,* 2009). The decreased activity of SOD in kidney in CCl_4-treated rats may be due to the enhanced lipid peroxidation or inactivation of the antioxidative enzymes. This would cause an increased accumulation of superoxide radicals which could further stimulate lipid peroxidation. Also, it may result in less scavenging of free radicals leading to generation of other forms of carbon-, nitrogen-and oxygen-centered radicals, which could lead to the lipid peroxidation, nitric oxide formation and decrease in GSH level found in kidneys after CCl_4 administration.

Our results are in agreement with the results that obtained by Tirkey *et al.* (2005) who have conducted experiments to determine the effect of CCl4 on the renal damages in rats.

Physalis extract recovered the activities of the antioxidant enzymes such as SOD, CAT, GST, GPx and GR in CCl_4-treated rats. The protective effects of physalis extract in maintaining the GSH level towards control have increased the capacity of endogenous antioxidant defense and increased the steady state of GSH and/ or its rate of synthesis that confers enhanced protection against oxidative stress.

The presence of abnormally high levels of urea, uric acid and creatinine in serum are possible indicators of hepatic and/or kidney injuries induced through CCl_4 treatment (Ogeturk *et al.,* 2005a). The serum creatinine level does not rise until at least half of the

kidney nephrons are damaged or destroyed (Bhattacharya *et al.,* 2005).

Khan *et al.* (2009) reported that chronic renal injuries and urea elevations developed in rats after CCl_4 intoxication.

The present study revealed that administration of physalis extract significantly restored the levels of urea, uric acid and creatinine in serum. Similar investigations were also documented that different plant extracts significantly recovered the renal injuries induced through CCl_4 intoxication (Ogeturk *et al.,* 2005a; Khan *et al.,* 2010).

The protective effects of *Physalis peruviana* extract against the CCl_4-induced renal injury could be attributed to its high levels of polyphenols and other antioxidants like flavonoids.

CCl_4-treated rats have shown characteristic morphological findings such as interstitial fibrosis, glomerular and tubular degeneration, interstitial mononuclear cell infiltration. The vasoconstriction induced by CCl_4 produces an ischemic local environment, which leads to a number of cellular damages such as deterioration in membrane integrity. The severe changes were not observed in the groups treated with physalis extracts suggesting the protective effects of physalis in attenuating CCl_4-induced morphological changes. Similar histopathological changes were observed by Ozturk *et al.* (2003) and Ogeturk *et al.* (2005a) in renal tissues of rats treated with CCl_4.

It is believed that with these histopathological changes the capacity of tubular absorption may have been altered, thus bringing about functional overload of nephrons with subsequent renal dysfunction (Adewole *et al.,* 2007).

Immunohistochemical investigation has shown that the immunostaining activity for Bcl-2 was decreased in CCl_4 group indicating the apoptotic effect of CCl_4. The protective effect of physalis extract was shown in rats treated with the extract where the numbers of Bcl-2 immunostaining cells were increased indicating the anti-apoptotic effect of physalis extract.

The regulation of apoptosis is another potential mechanism through which many agents such as flavonoids may prevent toxicity and cancer. Noteworthy, consequences from the toxin-induced excessive oxidative stress, depletion of antioxidant enzymes and induction of membrane lipid peroxidation may prompt the extrinsic or intrinsic apoptotic pathways. These pathways eventually lead to the activation of caspases pathway for apoptosis that ends up with caspase-3 activation, the real executioner of apoptosis once triggered, the active caspases initiated cell apoptosis (Guicciardi and Gores, 2005; Hassan *et al.,* 2011). Additionally, Cai *et al.* (2005) found that CCl_4 administration led to cytochrome c release from mitochondria after 4 h. It is well known that cytochrome c released from mitochondria into the cytosol triggers the activation of caspase-9 and caspase-3 in the mitochondrial pathway. In view of this, we propose the involvement of the mitochondrial pathway via caspase-3 activation in CCl_4-induced apoptosis.

We suggest that the induction of antioxidant enzymatic and non-enzymatic defense systems and suppression of MDA and NO by physalis extract could be effective in preventing apoptosis activation by caspase cascades triggered by CCl_4 which might be supported by previous finding (El-Mahdy et al., 2008; Hassan et al., 2011).

In the present study, the rats treated with CCl_4 showed a decrease in body weight and hence increased relative kidney weight. Chidambara Murthy *et al.* (2002) suggested that CCl_4-induced weights loss might be due to gastrointestinal toxicity and by reduced ingestion of food.

It has been noticed that many of plants which are rich in phenolic compounds and flavonoids, are widely used as antioxidant and antimutagenic (Sivalokanathan *et al.,* 2006).Various chemical compounds like 28-hydroxywithanolide, withanolides, phygrine, kaempferol, and quercetin di- and tri-glycosides are reported to be present in *Physalis peruviana* (Arun and Asha, 2007).

The observed effects of the extract could be related to chemically defined compounds. Flavonoids show their antioxidative action through scavenging or chelating process. Phenolic content is also important because of the presence of hydroxyl groups possessing scavenging ability. It can, therefore, be speculated that the observed antioxidant effects of physalis extract could be due to the presence of flavonoids and phenolic contents.

The protective effects of physalis peruviana extract against CCl_4 – induced renal injury can be explained on the basis of its nutritional composition. It contains biologically active components e.g. physalins, withanolides, phytosterols and polyunsaturated fatty acids e.g. linoleic acid and oleic acid. Among its major components are high amounts of vitamins A,B and C as well as the presence of essential minerals, magnesium, calcium, potassium, sodium and phosphorus which are classified as macronutrients, while the Iron and Zinc, for example, are considered as micronutrients (Szefer & Nriagu, 2007).

According to Wu *et al.* (2005) Zinc is a mineral that acts as a nonenzymatic antioxidant, so that its consumption prevents oxidative damage of the cell.

β-carotene has antioxidant activity that deactivates free radicals generated in tissues (Castro *et al.,* 2008).

Vitamin C is an important dietary antioxidant, since it reduces the adverse effects of reactive oxygen and reactive nitrogen that can cause damage to

macromolecules such as lipids, DNA and proteins, which are related to cardiovascular disease, cancer and neurodegenerative diseases (Naidu, 2003).

In conclusion, the present results revealed that *Physalis peruviana* extract alleviates the nephrotoxicity induced by CCl_4 in albino rats. The protective effects of *Physalis peruviana* are performed through multiple ways. *Physalis peruviana* scavenges free radicals that are produced by CCl_4, increases the activity of antioxidant-defense system and a greater susceptibility of the kidney to oxidant stress might be anticipated. Therefore, physalis extract may be used as a potential dietary antioxidant to retard aging and preventing diseases caused by ROS or ameliorating oxidative damage in tissues.

Conflict of interest statement

The authors declare that there are no conflicts of interest.

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5. **Refereneces**

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