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Nephro-protective effect of (*Arachis hypogaea* L.) peanut skin extracts on CCl_4 induced kidney damage in mice

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Abstract

The study aimed to assessment the antioxidant activity of peanut (*Arachis hypogaea*) skin extracts in male albino mice with administrated intraperitoneally 3 ml/kg CCl_4 to induce nephrotoxicity. The phytochemical analysis of the peanut skin extracts were investigated, the result showed presence of flavonoids, phenols, alkaloids, glycosides and tannins in methanolic extract, while alkaloids were not detected in aqueous extract. In addition, individual phenolic composition of peanut skin was analyzed by HPLC method which showed 7 phenolic compounds were detected (chlorogenic acid, caffeic acid, epicatechin, *p*-coumaric acid, quercetin, luteolin and kaempferol) in methanolic extract. The nephro-protective effect of peanut skin extract was evaluated in CCl_4 induced renal toxicity. The experiment was conducted in two methods: pre-treatment groups and post-treatment groups. Mice were treated with 50 and 100 mg/kg of aqueous and methanolic peanut skin extracts for 35 days before being damaged by CCl_4 (pre-treatment group), and the other groups (post-treatment groups) which the mice were injected with CCl_4 and received 50 and 100 mg/kg of aqueous and methanolic peanut skin extracts for 35 days. Biochemical studies showed that there is decrease in the levels of serum blood urea and creatinine while increases in the levels of albumin with significant differences ($p < 0.01$) when compared with the CCl_4 treated group. The histopathological examination of kidney obtained from mice with administrated intraperitoneally 3 ml/kg CCl_4 showed histopathological changes in the kidney represented excessive accumulation of protein material inside the proximal convoluted epithelial cells, while when treated with 100 mg/kg of peanut extract revealed look like normal structure appearance but with certain degenerative changes of the renal tubular epithelium.

Keywords: Nephro-protective, CCl_4, phenolic compounds, HPLC, *Arachis hypogea* **L.**

INTRODUCTION

The kidney is a vital organ in the human body and is one of the most complicated organs in terms of both structure and function [1]. Renal toxicity is one of the most common kidney problems and especially occurs when the body is subjected to chemical reagents or drugs [2]. Carbon tetrachloride (CCl4) is one of the most potent toxins, commonly used as a chemical inducer of experimental liver injury. In addition, many studies showed that CCl4 can induce kidney damage, which is widely used in scientific research to produce experimental model that mimic the oxidative stress in many pathophysiological situation [3]. The antioxidants are the first line of choice to take care of oxidative stress , endogenous antioxidant defenses include a network of compartmentalized antioxidant systems are necessary for sustaining life by maintaining a delicate intracellular redox balance and minimizing undesirable cellular damage caused by reactive oxygen species (ROS) the same antioxidant just due to its free radical scavenging activity may act as disease promoter, by neutralizing the physiologically desired ROS molecules, and as disease alleviator by removing the excessive levels of ROS species [4].

Arachis hypogaea L. is an annual herbaceous plant [5], belongs to the Leguminosae family [6]. Peanut seed has high nutritional and commercial values due to the presence fatty acids, vitamins, carbohydrates, calcium and phosphorus [7]. Peanut skins are regarded as a low economic value by-product of the peanut industry; Peanut skin could provide an inexpensive source of natural antioxidants, such as catechins and procyanidin, for use in food and dietary supplements. Also it contains higher concentration of procyanidin trimers and tetramers than grape seed which gives peanut skin a comparative advantage as a source of potent antioxidants [8]. The aim of this study is to determine the role of *Arachis hypogaea* L. skin as nephro-protective against CCl_4 induced nephro-toxicity in mice.

MATERIALS AND METHODS

Chemical substances

All reagents were of the highest purity available. CCl_4 were purchased from Sigma-Aldrich (Germany). Standard phenolic compounds clorogenic acid, quercetin, luteolin, *p*-coumaric acid and kaempherol were bought from company (sigma, USA). Commercial kits of Urea, Creatinine and Albumin were bought from company (Agappe, Swiss).

Plant collection

Raw peanuts pods were collected from the local Iraqi markets. Pods were manually shelled and the skins were collected from the raw peanut kernels. The skins were ground using a grinder and stored at -20°C for further analysis.

Preparation of aqueous extract

Water extract was prepared according to [9]. Macerated 100 gram of peanut skin in 1000 ml of distilled water for 72 hours, after extraction, the mixture was vacuum filtered through Whitman No. 1 paper and the filtrate was dried at 50°C by a rotary evaporator. The resulting extract stored in amber glass vials at 4 °C until analyzed. The whole process was completed under dim light to minimize light induced degradation of phenolics, which are generally light sensitive.

Preparation of methanolic extract

Methanolic extract was prepared according to [10] by using Soxhelt apparatus. So 50 gram of peanut skin was put in a thimble and 350 ml of methanol was added within 40-

60 ^{0}C for 6 hours. The solution have been filtered through a filter paper Whitman No.1 and evaporated to dryness under vacuum at 40°C, the dried extract have been weighed and stored in amber glass vials at 4 °C until analyzed.

Phytochemical screening of peanut skin extracts

Phytochemical test of peanut skin extracts were done according to [10, 11, 12, 13].

High-Performance Liquid Chromatography (HPLC)

Peanut methanolic and aqueous extracts were identified by (HPLC) according to [14], under the following conditions as shown in table (1).

Table 1. Conditions on HPLC peanut extracts

Pump model	S 2100 Quaternary Gradient Pump
The mobile phase (A)	(Methanol : D.W : acetic acid) (85 : 13 : 2)
The mobile phase (B)	(Methanol : D.W : acetic acid) (25 : 70 : 5)
Column	C18-ODS
Column dimension	25 cm × 4.6 mm
Detector UV	360 nm
Flow rate	1ml/min
Sample volume	20 µl
Temperature	25°C

Fourier transform infrared (FTIR) assay

Fourier Transform Infrared spectroscopy is a technique based on the vibrations of the atoms of a molecule, an infrared spectrum is commonly obtained by passing infrared radiation through a sample and determining what fraction of the incident radiation is absorbed at a particular energy. The FTIR spectrum was recorded between 4000 and 400 cm^{-1}[15].

Experimental animals

Forty male albino mice weighing 35 ± 5 g were obtained from Biotechnology Research Center, AL- Nahrain University. They were kept in standard conditions, the temperature about 22 °C, 12 hours light/dark cycle. The forty mice were randomly divided into ten groups of four animals each. The experiment was conducted in two methods: **pre-treatment** groups and **post-treatment** groups.

Group 1: This group served as a negative control in which the mice received normal feed and distilled water for 35 days

Group 2: This group was a positive control for CCl_4, which induce liver and kidney damage in mice. CCl_4 was solved in olive oil with ratio (1:3) (CCl4: olive oil) at a dose of 3 ml/kg injected intraperitoneally (i.p.).

Group 3: This group was the **pre-treatment** group, in which the mice were administered with 50 mg/kg methanolic extract orally for 35 days and injected (i.p.) 3 ml/kg of CCl_4 and olive oil mixture on the 35 day.

Group 4: This group was the **pre-treatment** group, in which the mice were administered with 100 mg/kg methanolic extract orally for 35 days and injected (i.p.) 3 ml/kg of CCl_4 and olive oil mixture on the 35 day.

Group 5: This group was the **pre-treatment** group, in which the mice were administered with 50 mg/kg aqueous extract orally for 35 days and injected (i.p.) 3 ml/kg of CCl_4 and olive oil mixture on the 35 day.

Group 6: This group was the **pre-treatment** group, in which the mice were administered with 100 mg/kg aqueous extract orally for 35 days and injected (i.p.) 3 ml/kg of CCl_4 and olive oil mixture on the 35 day.

Group 7: This group was the **post-treatment** group in which the mice were injected (i.p.) 3 ml/kg of CCl_4 and olive oil mixture on the 1st day and treatment with 50 mg/kg methanolic extract orally for 35 days.

Group 8: This group was the **post-treatment** group in which the mice were injected (i.p.) 3 ml/kg of CCl_4 and olive oil mixture on the 1st day and treatment with 100 mg/kg methanolic extract orally for 35 days.

Group 9: This group was the **post-treatment** group in which the mice were injected (i.p.) 3 ml/kg of CCl_4 and olive oil mixture on the 1st day and treatment with 50 mg/kg aqueous extract orally for 35 days.

Group 10: This group was the **post-treatment** group in which the mice injected (i.p.) with 3 ml/kg of CCl_4 and olive oil mixture on the 1st day and treatment with 100 mg/kg aqueous extract orally for 35 days.

Collection of blood

Blood samples were collected at the end of the experiment; the mice were anesthetized with the injection of 200 µl (160 µl ketamine 10% + 40 µl xylazine) of anesthesia agent. Then their abdominal areas were opened and the blood samples were directly taken from their hearts. The blood sample was rocked slightly and centrifuged at 3000 rpm for 5 minutes. The serum was then stored in the freezer at -21^{o}C until analyzed [16].

Statistical Analysis

The Statistical Analysis System (SAS) program was used to effect of difference factors in study parameters. Least significant difference –LSD test (ANOVA) was used to significant compare between means in this study [17].

RESULTS AND DISCUSSION

Phytochemical screening of peanut skin extracts

Phytochemical screening means the extraction, identification and screening of the medicinally active substances found in plants, some of the bioactive substances that are derived from plants are flavonoids, alkaloids, carotinoids, tannin, antioxidants, and phenolic compounds [18]. Different phytochemicals have a wide range of activities that may help in protection against various diseases [19]. The aqueous and methanolic extracts of the peanut skin extracts were subjected to different chemical tests for the detection of different phyto constituents in (Table 2) by using standard procedures. The results showed that the aqueous and methanolic extracts contain (Flavonoids, Phenols, Tannins and Glycosides) While the presence of alkaloids in the aqueous extract was not detected.

Table 2. Detection of some active compounds in peanut skin extracts

Phytochemical compound		Aqueous Extract	Methanolic Extract	Result
Flavonoids		+	+	yellow color
Phenols	ferric chloride	+	+	bluish green color
	lead acetate	+	+	reddish brown precipitate
Alkaloids	Dragendorff test	-	+	reddish brown precipitate
	Meyer test	-	+	White precipitate
Tannins		+	+	white gelatin
Glycosides		+	+	violet ring
Saponine		+	-	thick foam

+ Positive, - negative.

High-performance liquid chromatography (HPLC)

Phenolic compounds of peanut skin were analyzed by HPLC according to [14] method. In this study, 5 phenolic compounds were detected (chlorogenic acid, quercetin, luteolin, *p*-coumaric acid and kaempferol) in the methanolic extract as shown in (Figure 1) when compared with standard compounds as shown in (Figures 2). The results were in agreement with the results of Mar *et al*. [20], who reported that peanut skin contained relatively higher amounts of phenolic compounds such as (*p*-hydroxybenzoic acid, chlorogenic epicatechin, *p*-coumaric acid, ferulic acid, resveratrol and quercetin). Furthermore, Al-Jubouri [21] determined 10 phenolic compounds such as (chlorogenic acid, caffeic acid, epicatechin, *p*-coumaric acid, ferulic acid, resveratrol, quercetin, daidzin, luteolin and kaempferol) in methanolic peanut skin.

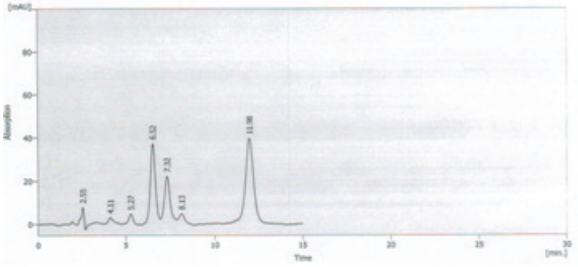

Figure 1. HPLC chromatogram of phenolic compounds in methanolic peanut skin

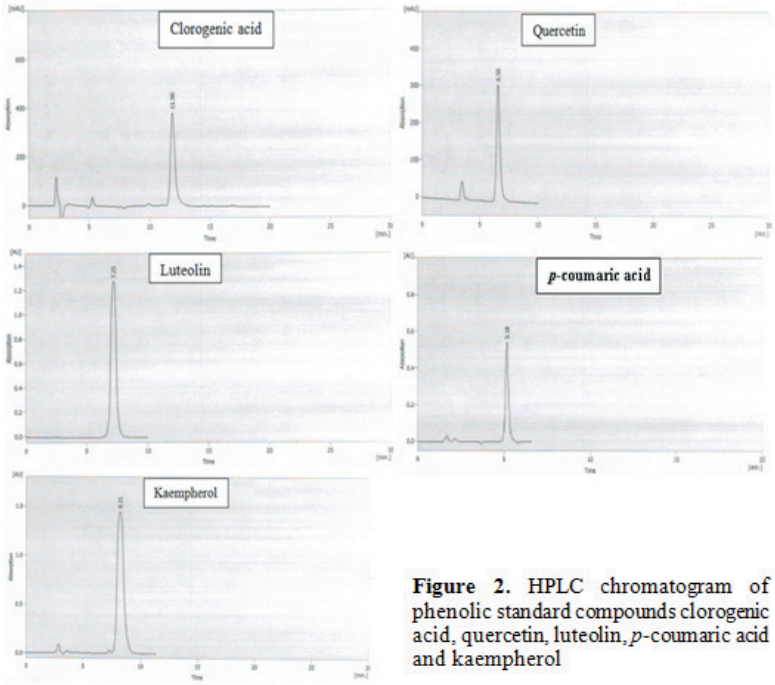

Figure 2. HPLC chromatogram of phenolic standard compounds clorogenic acid, quercetin, luteolin, *p*-coumaric acid and kaempherol

Fourier Transform Infra-Red (FTIR)

Fourier Transform Infra-Red stands for Fourier Transform Infrared, the preferred method of infrared spectroscopy. In infrared spectroscopy, IR radiation is passed through a sample. Some of the infrared radiation is absorbed by the sample and some of it is passed through (transmitted), the resulting spectrum represents the molecular absorption and transmission, creating a molecular fingerprint of the sample. Like a fingerprint no two unique molecular structures produce the same infrared spectrum, this makes infrared spectroscopy useful for several types of analysis [22]. Results of the FTIR Spectra of the methanolic and aqueous extracts of Peanut skin revealed the presence of different functional groups such as Phenolic– OH group stretching, C-H stretching, Aromatic C=C and Aliphatic C–O (Table 3). Figure (3 and 4) shows the infrared spectra for the aqueous and methanolic peanut skin extracts.

Table 3. The IR Frequencies region for the functional groups of peanut skin extracts

The functional groups	I.R Frequencies standard groups (cm-1)	I.R. Frequencies of aqueous extract	I.R. Frequencies of methanolic extract
Phenolic OH group stretching	3650-2500	3344.57	3360.00
C-H stretching	2960-2850	2947.23	2947.23
Aromatic C=C	1680-1620	1651.07	1647.21
Aliphatic C-O	1300 -1000	1029.99	1018.41

Zavoi *et al*., [23] investigated the hepatoprotective action using FTIR analysis of polyphenolic composition of medicinal herbs viz., *Cynara scolimus*, *Taraxacum officinalis*, *Chelidonium majus*, *Hypericum perforatum*, *Silybum marianu* and *Lycopodium clavatu*) from the wild flora of Romania. Thenmozhi *et al*., [24] screened compounds in *Eclipta* species by using FTIR and HPLC. Khoddami *et al*., [25] analyzed different techniques for plant phenolic compounds. Sivakumar [26] attempted a phytochemical study, FT-IR, GC-MS analysis of *Solanum torvum* methanolic leaves extract.

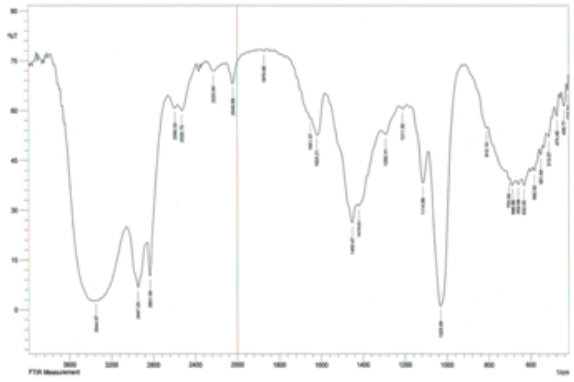

Figure 3. The infrared spectrum of aqueous extract

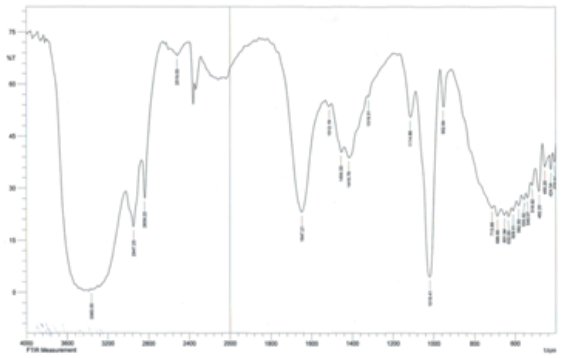

Figure 4. The infrared spectrum of methanolic extract

Protective and therapeutic role of the peanut skin extracts on kidney function enzymes

Effect of peanut skin Extracts on serum Urea level

Table (4) shows that the serum urea concentrations were significantly increased ($p < 0.01$) in the CCl_4 treated group (group 2) of mice for 4 weeks (37.47 ± 1.33 mg/dl) compared with the control group (group 1) (24.89 ± 1.05 mg/dl) indicating the induction of severe nephrotoxicity. Treatment with methanolic extract 50 mg/kg (Group 3) (31.08 ± 1.29 mg/dl) showed significant decrease ($p < 0.01$) in concentrations of serum urea compared with the CCl_4 treated group. Furthermore the levels of serum urea significantly decreased ($p<0.01$) in methanolic treated group 100 mg/kg (Group 4) (25.37 ± 1.26 mg/dl) and it was the best effective group when compared with the CCl_4 treated group, this indicate that the role of methanolic peanut extract as nephroprotective. Sherkatolabbasieh *et al*. [27] mention that the ethanolic extract of (*Allium saralicum* L.) 800 and 1600 µg/kg showed significantly decrease ($p \leq 0.05$) in concentrations of serum urea compared with CCl_4 treated group. Likewise when treatment with aqueous extract 50 mg/kg (Group 5) the result showed significant decrease ($p < 0.01$) in concentrations of serum urea (31.61 ± 1.00 mg/dl) when compared with CCl_4 treated group, also treatment with aqueous extract 100 mg/kg (Group 6) showed significant decrease ($p < 0.01$) in concentrations of serum urea (25.76 ± 1.28 mg/dl) compared with CCl_4 treated group.

The second method was the post-treatment, the results showed significant decrease ($p < 0.01$) in concentrations of serum urea (32.57 ± 0.93 and 25.49 ± 1.95 mg/dl) when treatment with methanolic extract 50 and 100 mg/kg (Group 7 and 8) respectively compared with the CCl_4 treated group. Furthermore treatment with aqueous extract 50 and 100 mg/kg (Group 9 and 10) showed significant decrease ($p < 0.01$) in concentrations of serum urea (35.47 ± 1.48and 30.64 ± 1.05 mg/dl) respectively when compared with the CCl_4 treated group. Ali *et al*. [28] mention that the flavonoid chrysin, which exerts strong antioxidant and anti-inflammatory activities, was tested on adenine-induced chronic kidney disease, chrysin, especially at 250 mg/kg, mitigated all manifestations of adenine-induced renal dysfunction, improved creatinine clearance, and reduced concentrations of urea in rats.

Effect of peanut skin extracts on serum Creatinine level

The level of serum Creatinine was significantly increased ($p <0.01$) in the CCl_4 treated group (group 2) of mice (1.350 ± 0.03 mg/dl) when compared to the control group (group 1) (0.803 ± 0.03 mg/dl) indicating the induction of severe nephrotoxicity. Treatment with methanolic extract 50 mg/kg (Group 3) showed significant ($p < 0.01$) decrease in concentrations of serum Creatinine (1.020 ± 0.02 mg/dl) when compared with the CCl_4 treated group. Furthermore the level of Creatinine significantly increased ($p<0.01$) in methanolic treated group 100 mg/kg (Group 4) (0.873 ± 0.03 mg/dl) when compared with the CCl_4 treated group. Kalantari *et al*. [29] mention that pre-treatment with (*Allium jesdianum* L.) hydroalcoholic extract at doses 500, 1000 and 2000 mg/kg caused a significant decrease ($P < 0.001$) in the level of serum creatinine. On the other hand treatment with aqueous extract 50 mg/kg (Group 5) showed significant decrease ($p < 0.01$) in concentrations of serum creatinine (1.006 ± 0.01 mg/dl) when compared with CCl_4 treated group (1.350 ± 0.03 mg/dl). Furthermore treatment with aqueous extract 50 mg/kg (Group 5) (0.903 ± 0.012 mg/dl) showed significant decrease ($p < 0.01$) in concentrations of serum creatinine compared with CCl_4 treated group as shown in (Table 4). Yoshioka *et al*. [3] showed that Zn (as ZnSO4) 50 mg/kg daily intake showed significantly decreased in creatinine and blood urea nitrogen levels and having protective effect from the ability of Zn to serve as an inducer of metallothionein (a known endogenous scavenger of free radicals) protects mice from acute nephrotoxicity induced by CCl_4.

The second method was the post-treatment, the results showed significant decrease ($p < 0.01$) in concentrations of serum creatinine (1.076 ± 0.02 and 0.913 ± 0.04 mg/dl) when treatment with methanolic extract 50 and 100 mg/kg (Group 7 and 8) respectively compared with the CCl_4 treated group. Furthermore treatment with aqueous extract 50 and 100 mg/kg (Group 9 and 10) showed significant decrease ($p < 0.01$) in concentrations of serum creatinine (1.106 ± 0.01and 0.967 ± 0.02 mg/dl) respectively when compared with the CCl_4 treated group as shown in (Table 4). Zangeneh *et al*. [30] suggest that (*Glycyrrhiza glabra* L.) aqueous extract at doses 30, 90, and 270 mg/kg (especially 270) could significantly ($p \leq 0.05$) reduce the raised levels of urea and creatinine as compared to the untreated group.

Effect of Peanut Skin Extracts on Serum Albumin Level:

Table (4) shows that the serum albumin concentrations were significantly decreased ($p < 0.01$) in the CCl_4 treated group (group 2) of mice (3.51 ± 0.12 mg/dl) compared to the control group (group 1) (4.79 ± 0.12 mg/dl) indicating the induction of severe nephrotoxicity. Albumin is the most abundant protein in human plasma with remarkably diverse functions including antioxidant activity, buffering properties, binding and transport capacities for numerous substances (free fatty acids, various ions, NO, bilirubin, peptides, uremic toxins and drugs) [31]. Treatment with methanolic extract 50 mg/kg (Group 3) showed significant increase ($p < 0.01$) in concentrations of serum albumin (4.13± 0.19 mg/dl) compared with the CCl_4 treated group. Furthermore the level of albumin significantly increased ($p<0.01$) in methanolic treated groups 100 mg/kg (Group 3) (4.79 ± 0.13 mg/dl) when compared with the CCl_4 treated group. Likewise the result showed significant increase ($p < 0.01$) in concentrations of serum albumin when treatment with aqueous extract 50 and 100 mg/kg (Group 5 and 6) (4.07 ± 0.07 and 4.79 ± 0.12 mg/dl) respectively when compared with CCl_4 treated group (3.51 ± 0.12 mg/dl). Zhang *et al*. [32] mention the protective effects of two intracellular polysaccharide (HIPS) purified fractions (HIPS1 and HIPS2) of *Hericium erinaceus* having significant decreased ($P < 0.05$) in albumin levels in mice.

The second method was the post-treatment, the results showed significant decrease ($p < 0.01$) in concentrations of serum albumin (4.05 ± 0.18 and 4.36 ± 0.12 mg/dl) when treatment with methanolic extract 50 and 100 mg/kg (Group 7 and 8) respectively compared with the CCl_4 treated group. Furthermore treatment with aqueous extract 50 and 100 mg/kg (Group 9 and 10) showed significant decrease ($p < 0.01$) in concentrations of serum albumin (4.03 ± 0.04 and 4.43 ± 0.14mg/dl) respectively when compared with the CCl_4 treated group. This indicates the effective role of peanut extracts as nephrotherapeutic. Mazani *et al*. [33] administration 80 and 120 mg/kg of (*Tanacetum parthenium* L.) methanolic extract to rats after exposure to CCl_4, significantly increased ($p<0.001$) serum albumin when compared with CCl_4 control group. The results showed there are no significant differences ($P<0.01$) between pre- and post-treatment methods.

Table 4. Effect of peanut skin extracts on serum Urea, Albumin and Creatinine in CCl_4 induced nephrototoxicity

Groups	Mean ± SE		
	Urea (mg/dl)	**Albumin (mg/dl)**	**Creatinine (mg/dl)**
Group 1	24.89 ± 1.05 d	4.79 ± 0.12 ab	0.803 ± 0.03 g
Group 2	37.47 ± 1.33 a	3.51 ± 0.12 e	1.350 ± 0.03 a
Pre-treatment groups			
Group 3	31.08 ± 1.29 c	4.13± 0.19 cd	1.020 ± 0.02 cd
Group 4	25.37 ± 1.26 d	4.79 ± 0.13 ab	0.873 ± 0.03 fg
Group 5	31.61 ± 1.00 c	4.07 ± 0.07 cd	1.006 ± 0.01 cd
Group 6	25.67 ± 1.28 d	4.97 ± 0.08 ab	0.903 ± 0.01 ef
Post-treatment groups			
Group 7	32.57 ± 0.93 bc	4.05 ± 0.18 cd	1.076 ± 0.02 bc
Group 8	25.49 ± 1.95 d	4.36 ± 0.12 cd	0.913 ± 0.04 ef
Group 9	35.47 ± 1.48 ab	4.03 ± 0.04 d	1.106 ± 0.01 b
Group 10	30.64 ± 1.05 c	4.43 ± 0.14 bc	0.967 ± 0.02 de
LSD value	3.826 **	0.383 **	0.075 **
** (P<0.01). Means having with the different letters in same column differed significantly			

Group 1: Control, **Group 2:** CCl_4 (3 ml/kg), **Group 3:** Methanolic extract (50 mg/kg) + CCl_4, **Group 4:** Methanolic extract (100 mg/kg) + CCl_4, **Group 5:** aqueous extract (50 mg/kg) + CCl_4, **Group 6:** aqueous extract (100 mg/kg) + CCl_4, **Group 7:** CCl_4 + Methanolic extract (50 mg/kg), **Group 8:** CCl_4 + Methanolic extract (100 mg/kg), **Group 9:** CCl_4 + aqueous extract (50 mg/kg), **Group 10:** CCl_4 + aqueous extract (100 mg/kg).

Histological examination of the kidney The light microscopic examination by specific staining of kidney cells in control tissues showed normal structure appearance of glomeruli and renal tissue (proximal and distal convoluted tubules) (Figures 5). Kidney sections obtained from the CCl_4 group (group 2) showed normal structure appearance but with excessive accumulation of protein material inside the proximal convoluted epithelial cells (Figure 6). Cordeiro and Kaliwal [34] mention that kidney of the mice treated with CCl_4 at dose 2 ml/gm for 3 days showed glomeruli were small and atrophied, loosely arranged in Bowman's capsule .

The pre-treatment groups, the kidney sections obtained from the group 3 (methanolic extract 50 mg / kg + CCl_4), the results showed congestion, degenerative changes and necrosis of renal epithelial cells with mild inflammatory cells infiltration (Figure 7A), while treatment with (methanolic extract 100 mg / kg + CCl_4) in group 4 showed normal look structure appearance but with certain degenerative changes of the renal tubular epithelium (Figure 7B). Also

in other section showed congestion, degenerative changes and apoptosis of renal epithelial cells when treated with aqueous extract 50 mg / kg + CCl_4 (Group 5) as shown in (Figure 7C). While treatment with aqueous extract 100 mg / kg + CCl_4 (Group 6) showed look like normal structure appearance with congestion (Figure 7D). This result agreed with Pal *et al*. [35] which mention that treatment with *Pithecellobium dulce* L. extract at dose 200 mg/kg + CCl_4 showed a considerable improvement in kidney morphology in mice.

The post-treatment groups, the kidney sections obtained from the group 7 (CCl_4 + methanolic extract 50 mg / kg), the results showed normal looking appearance with accumulation of protein granules inside the renal epithelial cells of proximal convoluted tubules as shown in (Figure 8A) . In group 8 (treated with CCl_4 + methanolic extract 100 mg /kg) the histopathological examination showed normal look structure appearance with accumulation of proteins granules inside the epithelial cells of renal tubules (Figure 8B). The kidney sections of group 9 (CCl4 + aqueous extract 50 mg / kg) showed necrosis of renal tubules with heavy chronic inflammatory cells infiltration and hyaline cast (Figure 8C). While the histopathological examination of group 10 (CCl4 + aqueous extract 100 mg / kg) showed normal look structure appearance but with certain degenerative changes of the renal tubular epithelium as shown in (Figure 8D). This explanation was agreed with Abdulhameed *et al*. [36] which mention that treatment with (CCl_4+ aqueous extract 500 mg/kg) of ginger (*Zingiber officinale*) showed intact glomerules and normal convoluted tubules structure of kidney. The findings showed the potential use of the methanolic and aqueous extracts of *Arachis hypogea* in concentration 100 mg / kg as a novel therapeutically useful hepato-nephroprotective (protect liver and kidney from injuries) and hepato-nephrocurative (Cures the injured liver and kidney) agent on CCl_4 induced toxicity in male albino mice.

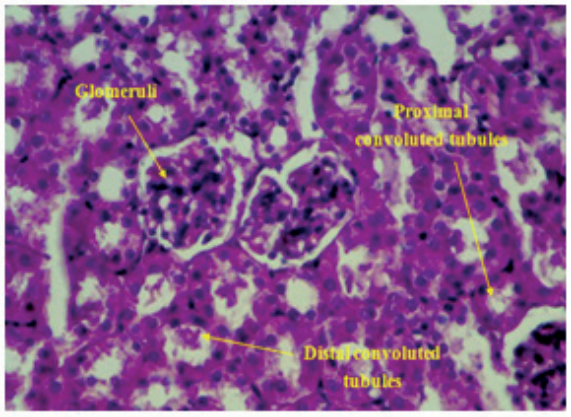

Figure 5. Histopathological section in the kidney of mice showing normal structure appearance of glomeruli and renal tissue (proximal and distal convoluted tubules) (H&E stain 400 X).

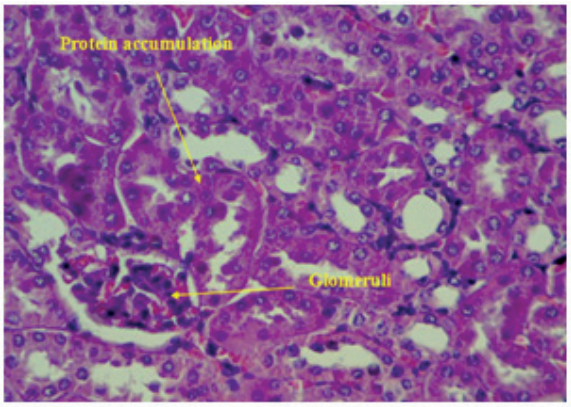

Figure 6. Histopathological section in the kidney of mice exposed to CCl_4 (3 ml / kg) showing look like normal structure appearance but with excessive accumulation of protein material inside the proximal convoluted epithelial cells (H&E stain 400 X).

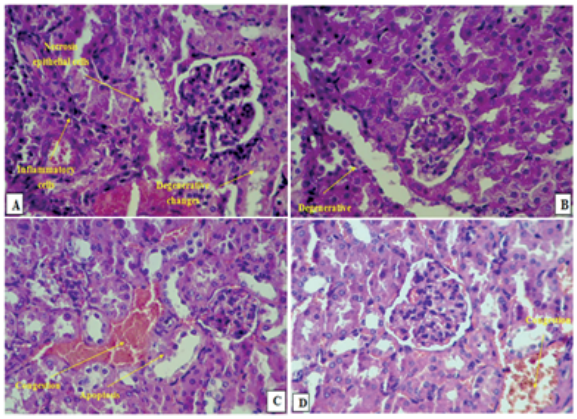

Figure 7. (A) Histopathological section showing congestion, degenerative changes and necrosis of renal epithelial cells with mild inflammatory cells infiltration. **(B)** Histopathological section showing look like normal structure appearance but with certain degenerative changes of the renal tubular epithelium. **(C)** Histopathological section showing congestion, degenerative changes and apoptosis of renal epithelial cells. **(D)** Histopathological section showing look like normal structure appearance with congestion (H&E stain 400 X).

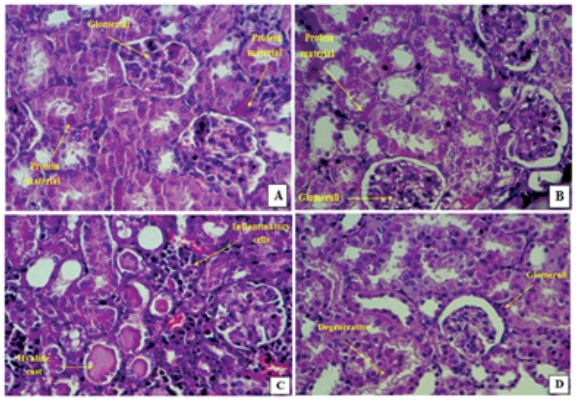

Figure 8. (A) Histopathological section showing normal looking appearance with accumulation of protein granules inside the renal epithelial cells of proximal convoluted tubules. **(B)** Histopathological section showing look like normal structure appearance with accumulation of proteins granules inside the epithelial cells of renal tubules. **(C)** Histopathological section showing necrosis of renal tubules with heavy chronic inflammatory cells infiltration i.e Pyelonephritis and hyaline cast. **(D)** Histopathological section showing look like normal structure appearance but with certain degenerative changes of the renal tubular epithelium (H&E stain 400 X).

CONCLUSIONS

These findings showed that *Arachis hypogaea* L. possessed active compounds such as flavonoids, phenols, alkaloids, glycosides and tannins. The use of *Arachis hypogaea* L. skin extracts was beneficial in attenuate CCl_4 induced renal toxicity by decrease in the levels of serum blood urea and creatinine. *Arachis hypogaea* L. has a novel therapeutic potential in kidney tissues in male albino mice, against oxidative damages on CCl_4.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

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REFERENCES

[01] T. Sakai, Recent topics in kidney research: morphology and molecular cell biology. *Anatomical Science International*, 92(2017), pp. 159–160.

[02] H. Nasri, M. J. Rafieian-Kopaei, Protective effects of herbal antioxidants on diabetic kidney disease. *Res Med Sci.,* 19(2014), pp. 82-83.

[03] H. Yoshioka, H. Usuda, N. Fukuishi, T. Nonogaki, S. Onosaka, Carbon tetrachloride induced nephrotoxicity in mice is prevented by pretreatment with zinc sulfate. *Biol. Pharm. Bull.,* 39 (2016), pp. 1042-1046.

[04] A. Rahal, A. Kumar, V. Singh, B. Yadav, R. Tiwari, K. Dhama, Oxidative Stress, Prooxidants, and Antioxidants: The Interplay. *Bio. Med. Research International*, (2014), pp. 1-12.

[05] D. E. Brann, Agronomy handbook. Virginia Cooperative Extension, Virginia Technology and Virginia State University, (2009), pp. 129.

[06] B. Cabanillas, U. Jappe, N. Novak, Allergy to peanut, soybean and other legumes: Recent advances in allergen characterization, stability to processing and IgE cross reactivity. *Mol. Nutr. Food Res*., 62(2018), pp. 1-9.

[07] C. Tuberoso, K. A. Kowalczy, E. Sarritzu, P. Cabras, Determination of antioxidant compounds and antioxidant activity in commercial oilseeds for food use. *J. Food Chem*., 103(2007), pp.1494-1501.

[08] J. Yu, M. Ahmedna, I. Goktepe, Chemical and physiologica1 properties of several sources of dietary fiber. *J. Food Sci*., 47(2006), pp. 1472.

[09] J. D. N'Guessan, A. P. Bidie, B. N. Lenta, B. Weniger, P. Andre, F. Guina, In vitro assays for bioactivity-guided isolation of anti-salmonella and antioxidant compounds in Thon ninja sanguine flowers. *Afr. J. Biotechnology*, 6(2007), pp. 1685-1689.

[10] American Association of Cereal Chemists (AACC), Method 08-01. The Association St. Paul, M.N., (1984).

[11] J. B. Harborne, Phytochemical Methods, A guide to modern techniques of plant analysis, Chapman and hall, London, New York. (1973).

[12] H. J. Jaffer, M. J. Mahmod, A. M. Jawad, A. Naji, A. AL-Naib, Phytochemical and biological screening of some Iraqi plants. Fitoterapia Lix 299. (1988).

[13] J. B. Harborne, Phytochemical methods,A guide to modern techniques of plant analysis. 3rd Edition, Chapman and hall: London. (1998).

[14] G. Mradu, S. Saumyakanti, M. Sohini, M. Arup, HPLC profiles of standard phenolic compounds present in medicinal plants. *IJPPR*., 4(2012), pp. 162-167.

[15] P. Singh, H. A. Andola, M. S. M. Rawat, G. J. N. Pant, V. K. Purohit, Fourier Transform Infrared (FT-IR) Spectroscopy in An Overview. *Research Journal of Medicinal Plants*, 5 (2011), pp. 127-135.

[16] T. P. Prohp, I. O. Onoagbe, Acute toxicity and dose response studies of aqueous and ethanol extracts of (*Triplochiton scleroxylonk* L.). *International Journal of Applied Biology and Pharmaceutical Technology*, 3(2012), pp. 400.

[17] Statistical Analysis System User's Guide. Statistical. Version 9.1th ed. SAS. Inst. Inc. Cary, N.C. USA. (2012).

[18] V. Pendyala, B. J. Ramesh, S. Vidyadhara, Phytochemical and pharmacological evaluation of Commiphora mukul for antidepressant activity in albino mice. *Asian J. Pharm. Clin. Res*., 10 (2017), pp. 360-363.

[19] K. Shanmugapriya, P. S. Saravana, P. Harsha, M. Peer, W. Binnie, A comparative study of antimicrobial potential and phytochemical analysis of *Artocarpus heterophyllus* and *Manilkara zapota* L. seed extracts. *J. Pharm. Res*., 4(2011), pp. 2587- 2589.

[20] M. W. Mar, A. Azizah, S. B. Bablishah, A. Farooq, S. P. Mohd, Phenolic compounds and antioxidant activity of peanut's skin, hull, raw kernel and roasted kernel flour. *Pak. J. Bot*., 43(2011), pp. 1635-1642.

[21] Z. H. H. Al-Jubouri, Antioxidant and Antibacterial Activity of Peanut (*Arachis hypogaea* L.) Skin Extracts. M.Sc. thesis, Genetic Engineering and Biotechnology Institute. University of Baghdad, (2017).

[22] S. Thomas, A. K. Zachariah, R. Kumar, M. Elsevier, Spectroscopic Methods for Nanomaterials Characterization. Illustrated. *Elsevier*, (2017), pp. 444.

[23] S. Zavoi, F. Fetea, F. Ranga, R. M. Pop, A. Baciu, C. Socaciu, Comparative fingerprint and extraction yield of medicinal herb phenolics with hepatoprotective potential, as determined by UV-Vis and FT-MIR spectroscopy. *Bot. Horti Agrobo*., 39(2011), pp. 82-89.

[24] M. Thenmozhi, P. K. Bhavya, S. Rajeshwari, Compounds identification using HPLC and FTIR in *Eclipta alba* L. and *Emilia sonchifolia* L. *International Journal of Engineering Science and Technology*, 3 (2011), pp. 292-298.

[25] A. Khoddami, A. Meredith, T. H. Wilkes, Techniques for Analysis of Plant Phenolic Compounds. *Molecules*, 18(2013), pp. 2328-2375.

[26] N. R. Sivakumar, Phytochemical Screening and GC-MS, FT-IR Analysis of Methanolic Extract Leaves of *Solanum torvum*. *International Journal of Research Studies in Biosciences*, 3(2015), pp. 61-66.

[27] H. Sherkatolabbasieh, L. Hagh-Nazari, S. Shafiezadeh, N. Goodarzi, M. M. Zangeneh, A. Zangeneh, Ameliorative effects of the ethanolic extract of (*Allium saralicum* L.) on CCl_4-induced nephrotoxicity in mice: A stereological examination. *Arch. Bio. Sci.,* 69(2017), pp. 535-543.

[28] B. H. Ali, M. Al Za'abi, S. A. Adham, J. Yasin, A. Nemmar, N. Schupp, Therapeutic effect of chrysin on adenine-induced chronic kidney disease in rats. *Cell. Physiol. Biochem*., 38(2016), pp. 248-257.

[29] H. Kalantari, M. D. Pajou, P. Kheradmand, M. Goodarzian, L. Zeidooni, Nephroprotective effect of hydroalcoholic extract *Allium jesdianum* L. boiss against carbon tetrachloride induced nephrotoxicity via stress oxidative in mice. *Pharmaceutical Sciences*, 24(2018), pp. 89-96.

[30] M. M. Zangeneh, A. Zangeneh, R. Tahvilian,

R. Moradi, Evaluation of the nephroprotective effect of (*Glycyrrhiza glabra* L.) aqueous extract on CCl_4 induced nephrotoxicity in mice. *Comparative Clinical Pathology*, 27(2018), pp. 1-8.

[31] G. Fanali, A. di Masi, V. Trezza, M. Marino, M. Fasano, P. Ascenzi, Human serum albumin: From bench to bedside. *Mol. Aspects Med*., 33(2012), pp. 209–290.

[32] C. Zhang, J. Li, C. Hu, J. Wang, X. Song, L. Jia, Antihyperglycaemic and organic protective effects on pancreas,liver and kidney by polysaccharides from Hericium erinaceus SG-02 in streptozotocin-induced diabetic mice. *J. Sci. Rep*., 7(2017), pp. 10847.

[33] M. Mazani, Y. Mahmoodzadeh, M. M. C. Asl, S. Banaei, L. Rezagholizadeh, A. Mohammadnia, Renoprotective effects of the methanolic extract of *Tanacetum parthenium* against carbon tetrachloride-induced renal injury in rats. *Avicenna J. Phytomed*., 8(2018), pp. 370-379.

[34] M. C. Cordeiro, B. B. Kaliwal, Protective role of bark extract of Bridelia retusa Spreng on CCl_4 induced histological toxicity in mice. *Journal of Pharmacognosy and Phytochemistry*, 2(2013), pp. 142-148.

[35] P. B. Pal, S. Pal, P. Manna, P. C. Sil, Traditional extract of *Pithecellobium dulce* fruits protects mice against CCl_4 induced renal oxidative impairments and necrotic cell death. *Pathophysiology*, 19(2012), pp. 101-114.

[36] I. S. Abdulhameed, D. F. Al-Mohamadamin, A. B. Abed, The effect of ginger plant (*Zingiber officinale* L.) aqueous extract on function and histological structure of kidney in mice treated with carbon tetrachloride. *International Journal of ChemTech Research*, 10(2017), pp. 208-219.