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Hepatoprotective effect of (*Arachis hypogea* L.) peanut skin extracts on CCl_4 induced liver damage in mice

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This study was carried out to evaluate the hepato-protective property of (*Arachis hypogea* L.) peanut skin extracts in CCl_4 induced hepatotoxicity in mice. The antioxidant activity was measured utilizing 2, 2-diphenyl-1-1 picrylhydrazyl (DPPH) radical scavenging capacity. The results showed that the methanolic extract was the highest free radical scavenging activity than the aqueous extract with values (92.34 ± 0.45 and 87.62 ± 0.44) respectively in 12 mg/mL compared to 89.61 ± 0.34 for Butylated hydroxytoluene (BHT) and 93.25 ± 0.06 for vitamin C, which means that the methanolic extract of peanut skin is superior to BHT. Furthermore, the total phenolic content was analyzed by using Folin-Ciocalteu method, the amount of total phenol in aqueous extract was15.32 ± 0.45, 39.29 ± 0.64 and 56.63 ± 1.03 mg/g in 2, 6 and 10 mg/ml respectively, while the methanolic extract was 47.08 ± 0.56, 68.40 ± 1.18 and 85.35 ± 0.62 mg/g respectively in the same concentrations. The hepato-protective effect of peanut skin extract was evaluated in CCl_4 induced hepato-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 show that there is decrease in the levels of serum ALT, AST, ALP, MDA and increases in the levels of SOD with significant differences ($p < 0.01$) when compared with the CCl_4 treated group. The histo pathological examination of liver obtained from mice with administrated intraperitoneally 3 ml/kg CCl_4 showed histopathological changes in the liver represented in fatty changes of excessive hepatocyte accumulation of fatty material, while when treated with 100 mg/kg of peanut extract revealed look like normal structure appearance of hepatic tissue and normal structure appearance but with few apoptotic cells.

Keywords: ***Arachis hypogea* L., Antioxidants,, phenolic compounds, CCl_4, hepato-protective.**

INTRODUCTION

The liver is an organ plays a central role in metabolism of nutrients, synthesis of glucose and lipids, and detoxification of drugs and xenobiotics (Chiang, 2014). Some chemicals or drugs can cause liver and kidney damage such as carbon tetrachloride (CCl_4), acetaminophen and cisplatin (Yamamotoya et al., 2017). Liver is not only the target organ of CCl4 which could be taken in the body via respiration, digestion, and the skin (Karakus et al., 2016). Oxidative stress (OS) term is used to describe an imbalance between the systemic manifestation of free radicals and capability of cells to detoxify them and negate

their damaging effects on proteins, lipid, and Deoxyribonucleic acid (DNA) (Chandra et al., 2015), the aim of many ongoing studies is to elucidate the underlying mechanisms and role of oxidative stress in disease onset and development. In particular, there is considerable emphasis on finding new therapeutic strategies for decreasing oxidative stress (Cabello-Verrugio et al., 2017).

Arachis hypogea L. is a rich in antioxidants as many fruits (Talcott et al., 2005). Peanut is a dietary source of biologically active polyphenols such as the stilbene trans resveratrol, flavonoids such as the proanthocyanidins, flavonols such as quercetin (Wang et al., 2008). Most of the antioxidants are located in the skin of peanuts (Lou et al., 2004). Peanut skin has been reported to be rich in polyphenols, and the color skin varies from light brown to deep red, the redness and hue angle of the peanut skin had strong correlations with total polyphenol content, they also had good correlations with antioxidant capacity supporting the strong correlation between total polyphenols and antioxidant capacity (Chukwumah *et al*., 2009). The aim of this study is to determine the role of *Arachis hypogea* L. skin as hepato-protective against CCl_4 induced hepatotoxicity in mice.

MATERIALS AND METHODS

Chemical substances

All reagents were of the highest purity available. DPPH, BHT and CCl_4 were purchased from Sigma-Aldrich (Germany). Commercial kits of AST, ALP, ALT were bought from company (Agappe, Swiss), (SOD) and (MDA) (cohesion biosciences, 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 N'Guessan et al., (2007). 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 (AACC, 1984) 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.

Evaluation of Antioxidant activity

DPPH assay

In order to obtain an indication of the antioxidant activity of peanut s skin crude extracts, 5 ml of a freshly prepared 0.004 % of 2,2-diphenyl-1-picrylhydrazyl (DPPH) in methanol was mixed with 50 µl of different concentration of peanut s skin crude extracts (2 , 4 , 6 , 8 , 10 and 12) mg/ml and the absorbance of each dilution, after 30 minutes, was measured at 517 nm (Kedare and Singh, 2011). Butylated hydroxytoluene (BHT) and vitamin C were the antioxidants used as positive control. All tests were performed in triplicate and the methanol was used as a blank solution. The percentage DPPH reduction (or DPPH radical scavenging capacity) was calculated as:

% Reduction = (Abs DPPH – Abs Dil.) /Abs DPPH x 100

Whereby: Abs DPPH = average absorption of the DPPH solution, Abs Dil. = average absorption of the three absorption values of each dilution.

With the obtained values, a graphic was made using Microsoft Excel. The EC50 of each extract (concentration of extract or compound at which 50% of DPPH is reduced) was taken from the graphic.

Determination of total phenolic contents

Total phenolic content of aqueous and methanolic extracts were determined spectrophotometrically using the Folin-Ciocalteu method described by Jayaprakasha et al., (2001) 0.4 ml of each sample were mixed with 2.0 ml of the Folin- Ciocalteu reagent (diluted 10 times), and 1.6 ml of 7.5% sodium carbonate solution. Total volume was adjusted to 5 ml by adding distilled water. The tubes were covered with parafilm and allowed to stand for 30 minutes at room temperature before the absorbance was

read at 760 nm spectrometrically. The tests were carried out in triplicate. Results were expressed as milligrams of gallic acid equivalent per gram of dry weight (mg GAE/g dry weight).

Determination of acute toxicity peanut skin extracts

A group of 40 adult normal male albino mice weighing 35±2 g were used for methanolic and aqueous peanut skin extract to study the acute toxicity. It was subdivided into six subgroups each of six mice. All subgroups were treated orally with rising doses of 100, 250, 500, 1000, 1500 and 2000 mg/kg of methanolic and aqueous peanut skin extract. Mortality rates were recorded 24 hours post treatment (El-Sayed et al., 2013).

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°C until analyzed (Prohp and Onoagbe, 2012).

Statistical Analysis

The Statistical Analysis System - SAS (2012) 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.

RESULTS AND DISCUSSION

Toxicity of Peanut Skin Extracts

Acute LD 50 is a statistically derived amount of a substance that can be expected to cause death in 50% of the animals when given by a specified route as a single dose and the animals observed for a specified time period (Adamson, 2016). In this study the result showed that no deaths occurred in either the aqueous or methanolic extract when treated by oral gavages at doses of 2000 mg/kg and the results revealed that the aqueous and methanolic extracts practically non toxic according to Hodge and Sterner (2005) as shown in (Table 1).

Table 1. Hodge and Sterner toxicity scale

No.	Term	LD50
1.	Extremely Toxic	Less than 1 mg /kg
2.	Highly Toxic	1 - 50 mg/ kg
3.	Moderately Toxic	50 - 500 mg/ kg
4.	Practically Non-Toxic	500 - 5000 mg /kg

Table 2. Radical Scavenging Activity of Peanut Skin Extracts

Concentration (mg/ml)	Mean ± SE			
	Methanolic Extract	Aqueous Extract	BHT	V.C.
2	70.80 ± 0.51 b	43.54 ± 1.08 d	71.57 ± 1.03 c	89.20 ± 0.11 c
4	80.63 ± 1.29 b	64.11 ± 1.17 c	85.23 ± 0.12b	90.75 ± 0.42 b
6	84.33 ± 0.69 ab	84.35 ± 0.50 b	88.73 ± 0.62a	92.60 ± 0.10 a
8	88.56 ± 0.54 ab	86.03 ± 0.67ab	89.35 ± 0.27a	92.84 ± 0.08 a
10	90.07 ± 0.21 a	87.54 ± 0.43 a	89.63 ± 0.39a	93.04 ± 0.35 a
12	92.34 ± 0.45 a	87.62 ± 0.44 a	89.61 ± 0.34a	93.25 ± 0.06 a
LSD value	8.047 **	2.396 **	1.696 **	0.731 **
** (P<0.01). Means having with the different letters in same column differed significantly				

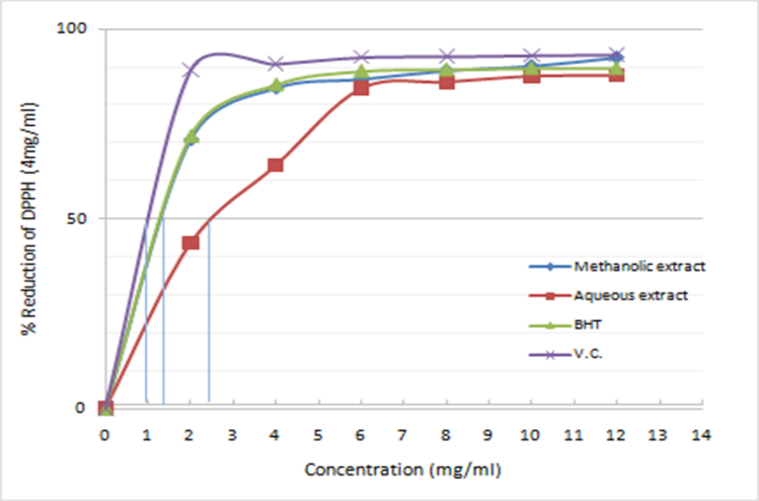

Figure 1. EC50 of peanut skin extracts

Antioxidant activity of peanuts skin extracts

DPPH assay

Flavonoids and phenolic compounds have proven their ability to deactivate or remove free radicals and able to protect lipids from being destroyed in the oxidative process (Frozza et al. ,2013). DPPH radical scavenging activity is one of the most widely used method for screening the antioxidant activity of plant extract (Sahu et al., 2013). In the DPPH assay, the antioxidants are able to reduce the stable radical DPPH to non-radical form DPPH-H. The purple colored of DPPH radical changes to yellow in the presence of a hydrogen donating antioxidant which could be measured at 517 nm. The use of the DPPH assay provides an easy and rapid way to evaluate antioxidants by spectrophotometer (Huang *et al*., 2005). In this study, the radical scavenging activity of the aqueous and methanolic extracts was compared at a concentration of (2, 4, 6, 8, 10 and 12) mg/ml. BHA and vitamin C were used as references. DPPH scavenging activity increased gradually with extract concentrations, as for the statistical analysis between different concentrations of the same extract, there was a significant difference at p<0.01 (Table 2). The

results showed that the methanolic extract was the highest free radical scavenging activity than the aqueous extract with values (92.34 ± 0.45 and 87.62 ± 0.44) respectively in 12 mg/mL compared to 89.61 ± 0.34 for BHT and 93.25 ± 0.06 for vitamin C, which means that the methanolic extract of peanut skin is superior to BHT. In agreement with this result, Adriano et al., (2011) revealed that peanut skin extracts showed a higher scavenging activity than that of BHA, which was used widely in the food industry as an antioxidant for a variety of food products. Gaafar et al., (2015) reported that methanolic extract of peanut skin showed higher activity compared to aqueous extracts. Also Al-Jubouri (2017) mention that the methanolic extract was the highest free radical scavenging activity in compare with ethyl acetate and aqueous extract. Furthermore, the antioxidant activity is expressed as an effective concentration (EC50). The half maximal effective concentration (EC50) are often refers to the concentration of a drug, toxicant or antibody which induces a response half way between the baseline and maximum after a specified exposure time, it commonly used as a measure of potency of a drug (Chan, 2015). In this study the radical scavenging capacity (EC50) of vitamin C was (1 mg/ml), while methanolic and BHT was found to be (1.5 mg/ml), On the other hand, the (EC50) of aqueous extract was (2.5 mg/ml) as shown in (Figure 1). Lee et al., (2008) mention that if the EC50 value of an extract is less than 10 mg/ml it indicates that the extract is an effective antioxidant. In this study, the EC50 value of peanut skin extracts were less than 10 mg /ml, and this indicates that the extracts were effective antioxidant.

Total phenolic content of peanut skin extracts

Phenolic compounds are a major class of semi water soluble compounds from fruit and vegetable sources (Rasoulia et al., 2017). They contain benzene rings, with one or more hydroxyl substituent, and range from simple phenolic molecules to highly polymerized compounds (Velderrain - Rodríguez et al., 2014). The peanut skin extracts were evaluated by using Follin-Ciocalteu´s reagent for the determination of total phenolic contents. The antioxidant activity of plant materials correlated well with the phenolic content (Murali *e*t al., 2013). In this study, the results showed that total phenolic content of aqueous extract was15.32 ± 0.45, 39.29 ± 0.64 and 56.63 ± 1.03 mg/g in 2, 6 and 10 mg/ml respectively, while the methanolic extract was 47.08 ± 0.56, 68.40 ± 1.18 and 85.35 ± 0.62 mg/g respectively in the same concentrations, as shown in (Table 3). Hoang et al., (2008) found that the amounts of phenolic compound in methanolic extract were much higher than in the case of the less polar solvents, and suggest that the methanol soluble fraction mostly consisted of phenolics compound. In agreement with AL-Azawi et al*.,* (2017) who mention that the highest amount of the total phenolics was shown by the methanolic extract more than aqueous and ethyl acetate extract. Yu et al., (2007) also found that one gram at dry peanut skin contained 90-125 mg of total phenolics and types of solvents used for extraction significantly affected the total phenolics content. The higher total phenolic content of peanut skin may be attributed to the presence of phenolic compounds (Yvonne et al., 2007). The total phenolic content of peanut skin found in this study was comparable to that reported by Wang et al., (2007). However, Nepote et al., (2005) reported that total phenolic content in peanut skin was 118 mg/g dry skin.

Effect of peanut skin extracts on serum Alanine Transaminase (ALT) Level

Table (4) shows that the serum (ALT) level was significantly increased ($p < 0.01$) in the CCl_4 treated group (group 2) of mice (61.36 ± 1.05 U/l) compared with the control group (31.99 ± 1.26 U/l) indicating the induction of severe hepatotoxicity. The toxic effect of CCl_4 may damage liver cells and other tissue cells and membranes through Lipid peroxidation of oxy and hydroxy radicals and other ways has been experimentally shown in *in vivo*/ *in vitro* environments (Karadeniz et al., 2009). CCl_4 is bio transformed by cytochrome P450 to the trichloromethyl free radical (CCl_3) that induces membrane lipid peroxidation and disturbs Ca^{2+} homeostasis to produce hepatocellular injury (Arun and Asha, 2007). When the animals treatment with methanolic extract 50 mg/kg (Group 3) the result showed significant decrease ($p < 0.01$) in concentrations of serum (ALT) (43.62 ± 0.69 U/l) compared with the CCl_4 treated group, as well as the levels of (ALT) significantly decreased ($p<0.01$) (34.61 ± 0.76 U/l) when treated with 100 mg/kg of methanolic extract (Group 4). Likewise the result showed significant decrease ($p < 0.01$) in concentrations of serum (ALT) (47.69 ± 1.26 U/l) when treatment with aqueous extract 50 mg/kg (Group 5) compared with CCl_4 treated group.

Table 3. Total Phenolic content of Peanut skin extracts

Peanut skin extracts	Concentration (mg/g)			LSD value
	2 mg/ml	6 mg/ml	10 mg/ml	
Methanolic extract	47.08 ± 0.56 C	68.40 ± 1.18 B	85.35 ± 0.62 A	5.271 **
Aqueous extract	15.32 ± 0.45 C	39.29 ± 0.64 B	56.63 ± 1.03 A	4.894 **
LSD value	1.994 **	3.746 **	3.348 **	---
**** (P<0.01).** Means having with the different letters in same row differed significantly				

Table 4. Effect of peanut skin extracts on serum AST, ALT, ALP in CCl_4 induced hepatotoxicity

Groups	Mean ± SE		
	ALT (U/l)	AST (U/l)	ALP (U/l)
Group 1	42.75 ± 1.51 c	31.99 ± 1.26 e	201.67 ± 1.99 d
Group 2	62.53 ± 1.26 a	61.36 ± 1.05 a	301.25 ± 7.82 a
Pre-treatment groups			
Group 3	51.64 ± 1.63 b	43.62 ± 0.69 b	212.13 ± 3.29 bcd
Group 4	43.91 ± 0.77 c	34.61 ± 0.76 de	208.08 ± 4.85 bcd
Group 5	52.64 ± 1.05 b	47.69 ± 1.26 b	218.17 ± 2.79 b
Group 6	46.82 ± 2.09 c	35.77 ± 1.81 cde	211.75 ± 1.13 bcd
Post-treatment groups			
Group 7	52.35 ± 1.81 b	43.62 ± 1.33 b	214.50 ± 2.86 bc
Group 8	44.21 ± 1.26 c	36.35 ± 1.26 cd	207.17 ± 1.99 cd
Group 9	55.55 ± 0.76 b	47.11 ± 2.80 b	216.79 ± 3.57 bc
Group 10	46.24 ± 2.01 c	38.97 ± 0.76 c	208.54 ± 3.21 bcd
LSD value	4.174 **	4.209 **	10.868 **
** (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).

Furthermore treatment with aqueous extract 100 mg/kg (Group 6) have significant decrease ($p < 0.01$) in concentrations of serum (ALT) (38.97 ± 0.76) compared with CCl_4 treated group. The decrease in the (ALT) levels in the groups treated with peanut skin extracts may be attributed to the presence of flavonoids which also have been known to possess significant hepatoprotective effect. Nquyen et al., (2017) mention that the presence of flavonoids have strongly linked to its hepatoprotective effect because flavonoid containing plant extracts help to stave off the hepatic damage caused by various noxious agents. The efficacy of any hepato-protective drug is mainly dependent on how effectively it reverses the toxic effects or restores normal hepatic physiological function (Rehman et al., 2018).

The second method was the post-treatment (7, 8, 9 and 10 groups), the results showed significant decrease ($p < 0.01$) in concentrations of serum (ALT) (43.62 ± 1.33 and 36.35 ± 1.26 U/l) 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 (ALT) (47.11 ± 2.80 and 38.97 ± 0.76 U/l) respectively when compared with the CCl_4 treated group. Pre- and post-treatment with (120 mg/kg) of (*Tanacetum parthenium* L.) could significantly ($P < 0.001$) decrease ALT, AST, ALP (Mahmoodzadeh et al., 2017).

Effect of peanut skin extracts on serum Aspartate Transaminase (AST) Level

The serum (AST) level was significantly increased ($p < 0.01$) in the CCl_4 treated group (group 2) of mice (62.53 ± 1.26 U/l) compared with the control group (group 1) (42.75 ± 1.51 U/l) indicating the induction of severe hepatotoxicity.

Treatment with methanolic extract 50 mg/kg (Group 3) showed significant decrease ($p < 0.01$) in concentrations of serum (AST) (51.64 ± 1.63 U/l) compared with the CCl_4 treated group. Furthermore the best effect in the level of (AST) was in methanolic treated groups 100 mg/kg (Group 4) (43.91 ± 0.77 U/l) when compared with the control group as shown in (Table 4). Chiu *et al*. (2018) mention that CCl_4 group have significant decrease in (AST) after treatment with ethanolic extract of *Polygonum orientale* L. (0.5 and 1.0 g/kg) and suggest that the activity might be partially associated with protocatechuic acid, taxifolin, and quercetin. On the other hand treatment with aqueous extract 50 mg/kg (Group 5) showed significant ($p < 0.01$) decrease in concentrations of serum (AST) (52.64 ± 1.05 U/l) when compared with the CCl_4 treated group. Furthermore the levels of (AST) significantly decreased ($p<0.01$) in methanolic treated groups 100 mg/kg (Group 6) (46.82 ± 2.09 U/l) when compared with the CCl_4 treated group. Borthakur *et al*. (2017) suggest that the decrease in serum AST levels in the aqueous extract of *Alternanthera sessilis* L. treated groups may be attributed to the presence of saponin, which exerts its hepatoprotective effect.

The second method was the post-treatment, the results showed significant decrease ($p < 0.01$) in concentrations of serum (AST) (52.35 ± 1.81 and 44.21 ± 1.26 U/l) 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 (AST) (55.55 ± 0.76 and 46.24 ± 2.01 U/l) respectively when compared with the CCl_4 treated group. AL-Mashhadani (2017) mention that 200 mg/kg fenugreek of (*Trigonella foenum graecum Linn* L.) leaves extract and 500 mg/kg fenugreek seed extract showed significant ($P<0.001$) differences in AST, ALT, ALP when compared to CCl_4 treated rats.

Effect of peanut skin extracts on Serum Alkaline Phosphatase (ALP) Level

Table (4) shows that the serum (ALP) level was significantly increased ($p <0.01$) in the CCl_4 treated group (group 2) of mice (301.25 ± 7.82 U/l) compared with the control group (group 1) (201.67 ± 1.99 U/l) indicating the induction of severe hepatotoxicity. Treatment with methanolic extract 50 mg/kg (Group 3) (212.13 ± 3.29 U/l) showed significantly decrease ($p < 0.01$) in concentrations of serum (ALP) when compared with the CCl_4 treated group. While the levels of (ALP) significantly decreased ($p<0.01$) in methanolic treated group 100 mg/kg (Group 4) (208.08 ± 4.85 U/l) and it was the best effective group when compared with the CCl_4 treated group. Mirazi et al., (2016) mention that treatment with hydroalcoholic extract of mangrove (*Avicennia marina* L.) leaves at doses of 400 and 800 ml/kg significantly ($P < 0.05$) decreased the serum levels of ALP in rats. Likewise the result showed significant decrease ($p < 0.01$) in concentrations of serum (ALP) when treated with aqueous extract 50 and 100 mg/kg (Group 5 and Group 6) (218.17 ± 2.79 U/l and 211.75 ± 1.13 U/l) respectively when compared with the CCl_4 treated group. Karakus et al., (2016) mention that (*Silybum marianum* L.) and (*Taraxacum officinale* L.) extracts at dose 100 mg/kg/day significantly ($p<0.05$) decreased the serum ALP. Moreover Amim et al., (2013) suggest the antioxidant property of the root of (*Taraxacum officinale* L.) attributed to possess important components such as lactones, triterpenes, sterols, flavonoids, and phenolic acids.

The second method was the post-treatment, the results showed significant decrease ($p < 0.01$) in concentrations of serum (ALP) (214.50 ± 2.86 and 207.17 ± 1.99 U/l) 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 (ALP) (216.79 ± 3.57 and 208.54 ± 3.21 U/l) respectively when compared with the CCl_4 treated group. This indicates the effective role of peanut extracts as hepatotherapeutic. A study showed that animals treated with CCl_4 with 250 mg/kg of (*Juniperus phoenicea* L.) berries caused a significant increase in ALP level because plant aqueous extract contained high levels of total phenolic compounds , tannins and flavonoids (Laouar et al., 2017).

Effect of peanut skin extracts on serum Superoxide Dismutase (SOD) Level

Superoxide Dismutase is a group of metalloenzymes that plays a crucial antioxidant role and constitutes the primary defense against the toxic effects of superoxide radicals in aerobic organisms (Kaur and Jindal, 2017).

Table 5. Effect of peanut skin extracts on serum SOD and MAD in CCl_4 induced hepatotoxicity

Groups	Mean ± SE	
	SOD (U/ml)	**MAD (nmol/ml)**
Group 1	19.11 ± 1.14 a	5.27 ± 0.63 d
Group 2	6.11 ± 0.43 e	14.18 ± 1.00 a
Pre-treatment groups		
Group 3	13.12 ± 0.59 cd	12.28 ± 0.31 b
Group 4	18.72 ± 1.75 a	7.48 ± 0.20 c
Group 5	13.93 ± 1.02 bcd	12.28 ± 0.31 b
Group 6	18.55 ± 1.33 a	8.06 ± 0.15 c
Post-treatment groups		
Group 7	13.82 ± 0.67 cd	12.46 ± 0.07 b
Group 8	17.06 ± 1.53 ab	8.04 ± 0.21 c
Group 9	12.97 ± 0.71 d	13.05 ± 0.69 ab
Group 10	16.33 ± 0.95 abc	8.83 ± 0.29 c
LSD value	3.230 **	1.409 **
** (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).

(Table 5) shows that the serum (SOD) level was significantly decreased ($p < 0.01$) in the CCl_4 treated group (group 2) of mice (6.11 ± 0.43 U/ml) compared with the control group (group 1) (19.11 ± 1.14 U/ml) indicating the induction of severe hepatotoxicity. Treatment with methanolic extract 50 mg/kg (Group 3) showed significantly increase ($p < 0.01$) in concentrations of serum (SOD) (14.12 ± 0.59 U/ml) compared with the CCl_4 treated group. Furthermore the levels of (SOD) significantly increased ($p<0.01$) in 100 mg/kg methanolic extract (Group 4) (18.72 ± 1.75 U/ml) and it was the best effective group compared with the CCl_4 treated group. Akdemir et al., (2017) Conclude that *p*-Coumaric acid (a phenolic class compound) protects the liver and kidney against Cisplatin-induced oxidative damage in rat and the SOD activities significantly $p < 0.05$ increased in (100 mg/kg) of *p*-Coumaric acid. On the other hand, treatment with aqueous extract 50 mg/kg (Group 5) showed significant increase ($p < 0.01$) in concentrations of serum (SOD) (13.93 ± 1.02 U/ml) when compared with CCl_4 treated group, while the level of serum (SOD) significantly increase ($p < 0.01$) (17.55 ± 1.33 U/ml) when treatment with aqueous extract 100 mg/kg (Group 6) compared with CCl_4 treated group. Lu et al., (2017) mention that the pre-treatment with phloretin at 100, 200 and 500 mg/kg significantly increased SOD activity against acute liver oxidative injury induced by CCl_4 in mice.

The second method was the post-treatment, the results showed significant decrease ($p < 0.01$) in concentrations of serum (SOD) (13.82 ± 0.67 and 17.06 ± 1.53 U/ml) 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 (SOD) (12.97 ± 0.71and 16.33 ± 0.95 U/ml) respectively when compared with the CCl_4 treated group. Tsai et al., (2017) mention the activities of antioxidant enzyme (SOD) significantly increased after treatment with (0.5 and 1.0 g/kg) ethanolic extract of *Coreopsis tinctoria* L. flowers.

Effect of peanut skin extracts on serum Malondialdehyde (MDA) Level

Malondialdehyde (MDA) is a commonly used biomarker for the assessment of lipid peroxidation (Mistry et al., 2013). (Table 5) shows that the serum Malondialdehyde (MDA) concentrations were significantly increased ($p <0.01$) in the CCl_4 treated group (group 2) of mice (14.18 ± 1.00 nmol/ml) compared with the control group (5.27 ± 0.63 nmol/ml) the increase of MDA level in the

CCl_4 treated group due to the increase of lipid peroxidation level (Messarah et al., 2013). Treatment with methanolic extract 50 mg/kg (Group 3) showed significant decrease ($p < 0.01$) in concentrations of serum (MDA) (11.07 ± 0.31nmol/ml) when compared with the CCl_4 treated group, while the best effect was in methanolic extract 100 mg/kg (Group 4) (7.48 ± 0.20 nmol/ml) with significant decreased ($p<0.01$) when compared with control group. Some compounds such as sabinene, tannins, and flavonoids can prevent lipid peroxidation and damage to cells (Ottu et al., 2013). Hussain et al., (2014) mention that *Alcea Rosea* extracts has antioxidant properties against free radicals due to having isoquercetin, kaempferol. Likewise when treatment with aqueous extract 50 mg/kg (Group 5) showed significant decrease ($p < 0.01$) (12.28 ± 0.31 nmol/ml) when compared with CCl_4 treated group. Furthermore treatment with aqueous extract 100 mg/kg (Group 6) showed significant decrease ($p < 0.01$) in concentrations of serum (MDA) (8.06 ± 0.15 nmol/ml) when compared with CCl_4 treated group. This indicates the effective role of methanolic peanut extract as hepatoprotective. Anand et al., (2011) mention that chrysin (natural flavonoid) at dose 200 mg/kg showed lower levels of MDA in rats, the ability of chrysin to enhance the levels of antioxidants along with its anti-lipid peroxidative activity and suggest that this compound might be potentially useful in counteracting the free radical mediated injury involved in the development of tissue damage caused by CCl_4.

The second method was the post-treatment, the results showed significant decrease ($p < 0.01$) in concentrations of serum (MDA) (12.46 ± 0.07 and 8.04 ± 0.21 nmol/ml) 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 (MDA) (13.05 ± 0.69 and 8.83 ± 0.29 nmol/ml) respectively when compared with the CCl_4 treated group. This indicates the effective role of peanut extracts as hepatotherapeutic. Eshak and Osman (2013) mention that treatment with 50 mg/kg body weight daily of (*Moringa oleifera* L.) leaves aquous extract was observed to have a therapeutic action against radiation hazards through enhancing of liver enzyme activities (AST, ALT and ALP), decreasing the malondialdehyde (MDA) in irradiated rats by gamma irradiation.

Histological examination of the liver

The light microscopic examination by specific staining of liver cells in control tissues (group 1) showed normal hepatic architecture with central vein and increase in kupffer cells (Figure 2). Liver sections obtained from the CCl_4 group (group 2) showed histopathological changes in the liver represented in fatty changes of excessive hepatocyte accumulation of fatty material (Figure 3). Chiu et al., (2018) mention that major changes in histology induced by CCl_4 resulted in increased coagulative necrosis, inflammation, and vacuolization when compared with the control group. The post-treatment groups, in which the mice were injected 3 ml/kg of CCl_4 on the 1st day and treatment with peanut skin extracts orally for 35 days. The results in the liver sections showed dispersed focal areas of necrosis and inflammatory cells, when treated with CCl_4 + methanolic extract 50 mg / kg (Group 7) as shown in (Figure 5A) . In group 8 (treated with CCl_4 + methanolic extract 100 mg /kg) the histopathological examination showed normal appearance structure with slight sinusoidal dilatation (Figure 5B). According to Al-Ezzy et al., (2017), the mice liver infected with CCl_4 and treated with 100 mg/ml of *Achillia melifolium* L. methanolic extract showed congestion with accumulation of glycoproteins granules, while mice liver that infected with CCl_4 and treated with 200 mg/ml of *Achillia melifolium* L. showed normal appearance structure, consist of central vein and threads of hepatocyte cells. Liver sections of group 9 (CCl4 + aqueous extract 50 mg / kg) showed congestion, dispersed areas of necrosis of hepatocyte cells and heavy inflammatory cells infiltration (Figure 5C). While the histopathological examination of group 10 (CCl4 + aqueous extract 100 mg / kg) showed depletion of glycoprotein granules inside the hepatocyte cells with preserve structure appearance of hepatic tissue as shown in (Figure 5D). Dutta et al., (2018) mention that histopathological evidences showed less leukocyte infiltrations sinusoidal dilations and bile duct proliferation when treated with CCl4 + 50 mg/kg of *Croton bonplandianus* L. extract compare with control group, while (CCl4 + 100 mg/kg) reflected comparatively less haemorrhagic necrosis and fatty infiltrations, furthermore, (CCl4 +250 mg/kg) demonstrated lowering of most of the injury signs

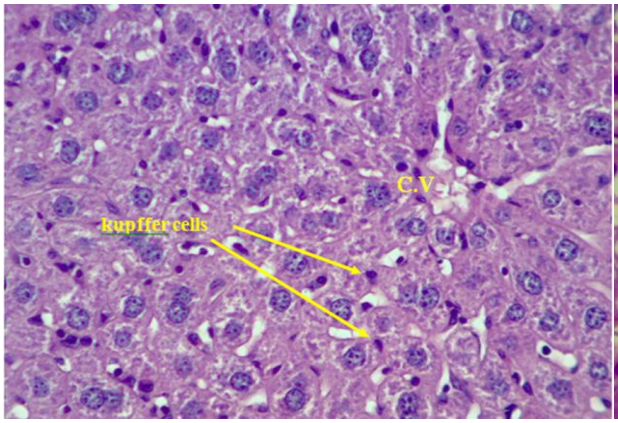

Figure 2. Histopathological section in the liver of mice showing normal looking structure appearance of hepatic tissue with central vein (C.V) and a thread like arrangement of hepatocyte cells and increase in kupffer cells (H&E stain 400 X).

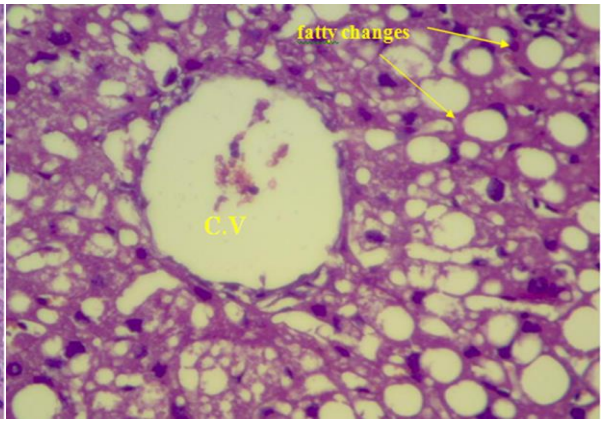

Figure 3. Histopathological section in the liver of mice exposed to CCl4 (3 ml / kg) showing fatty changes of excessive hepatocyte accumulation of fatty material (H&E stain 400 X).

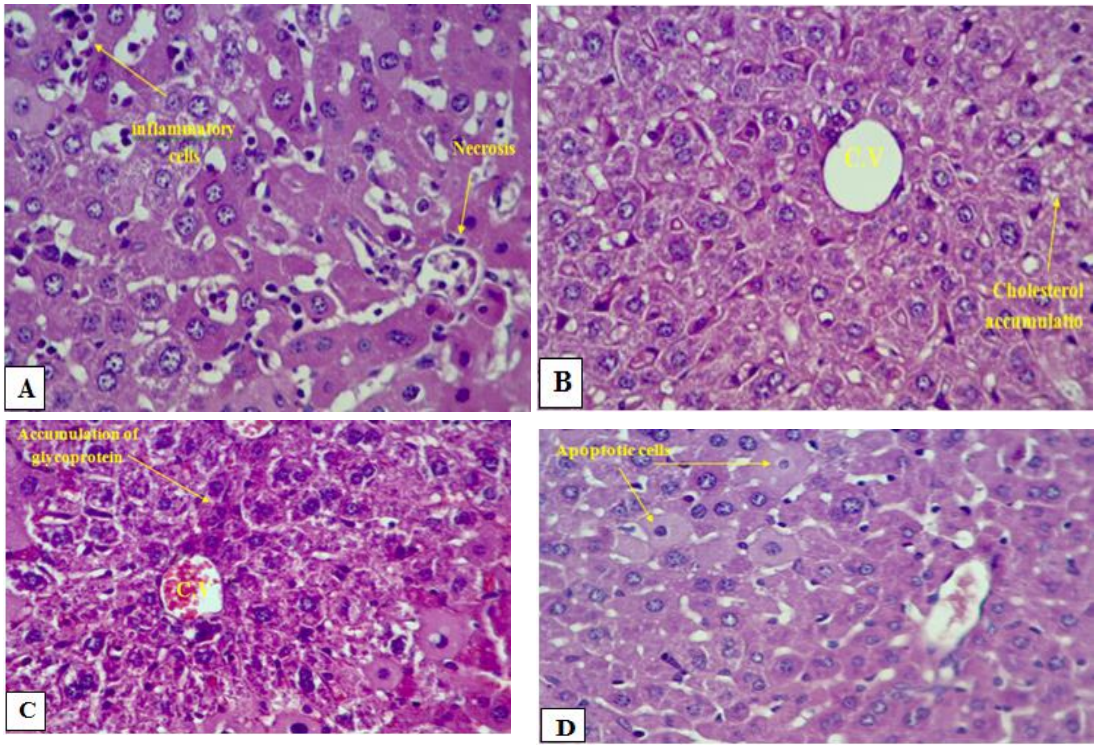

Figure 4. (A) Histopathological section in the liver of mice pre -treatment with methanolic extract 50 mg / kg for 35 days and exposed to CCl_4 (3 ml / kg) showing dispersed necrosis of hepatocyte cells with inflammatory cells infiltration. (B) Histopathological section in the liver of mice pre- treatment with methanolic extract 100 mg / kg for 35 days and exposed to CCl_4 (3 ml / kg) showing accumulation of cholesterol inside the hepatocyte cells. (C) Histopathological section in the liver of mice pre- treatment with aqueous extract 50 mg / kg for 35 days and exposed to CCl_4 (3 ml / kg) showing congestion, accumulation of glycoprotein granules with few apoptotic hepatocyte cells. (D) Histopathological section in the liver of mice pre- treatment with aqueous extract 100 mg / kg for 35 days and exposed to CCl_4 (3 ml / kg) showing look like normal structure appearance of hepatic tissue but with few apoptotic cells (H&E stain 400 X).

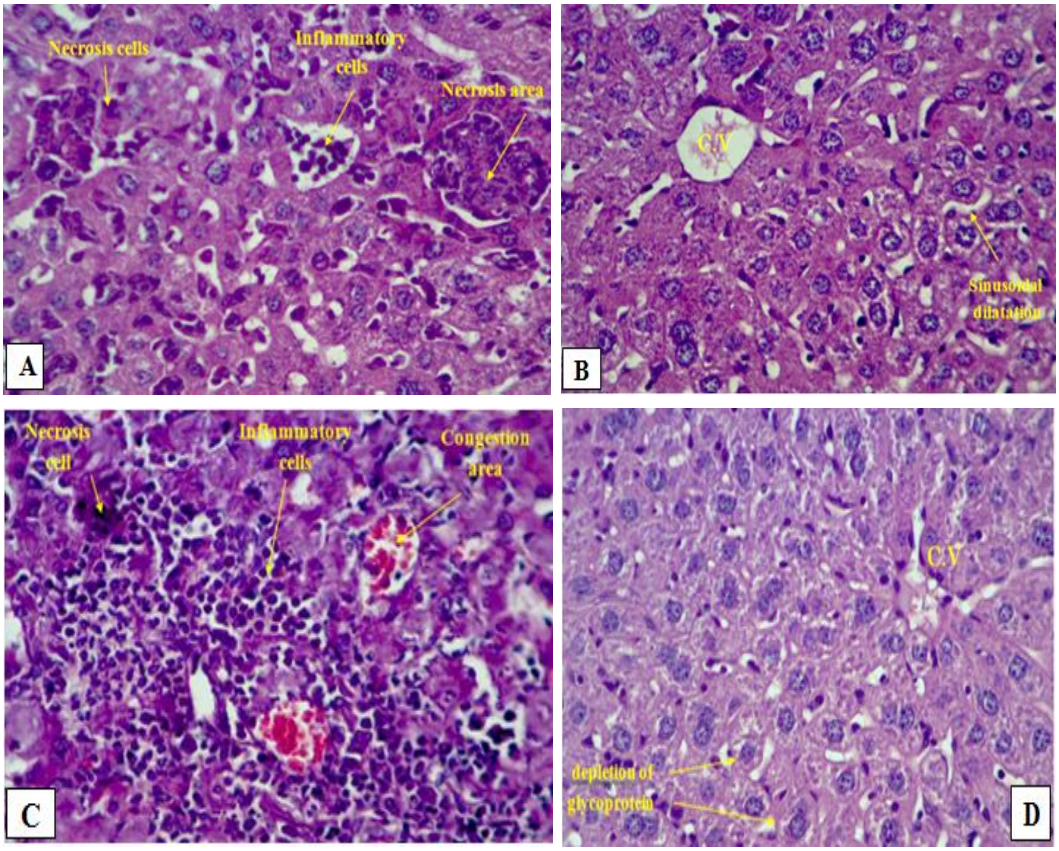

Figure 5. (A) Histopathological section in the liver of mice exposed to CCl_4 (3 ml / kg) and treatment with methanolic extract 50 mg / kg for 35 days showing dispersed focal areas of necrosis and inflammatory cells infiltration. (B): Histopathological section in the liver of mice exposed to CCl_4 (3 ml / kg) and treatment with methanolic extract 100 mg / kg for 35 days showing look like normal appearance structure with slight sinusoidal dilatation. (C): Histopathological section in the liver of mice exposed to CCl_4 (3 ml / kg) and treatment with aqueous extract 50 mg / kg for 35 days showing congestion, dispersed areas of necrosis of hepatocyte cells and heavy inflammatory cells infiltration. (D): Histopathological section in the liver of mice exposed to CCl_4 (3 ml / kg) and treatment with aqueous extract 100 mg / kg for 35 days showing depletion of glycoprotein granules inside the hepatocyte cells with preserve structure appearance of hepatic tissue (H&E stain 400 X).

CONCLUSION

These findings showed that *Arachis hypogaea* L. possessed strong antioxidant effects as free radical scavenger in quenching the DPPH. The total phenolic content of the methanolic extract was higher than aqueous extract. The use of *Arachis hypogaea* L. skin extracts was beneficial in attenuate CCl_4 induced hepto toxicity by modulating the activities of the antioxidant enzymes and lowering liver biochemical markers. *Arachis hypogaea* L. has a novel therapeutic potential in liver 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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AUTHOR CONTRIBUTIONS

AHA and AKI contributed to the design of the experiments and performed the experimental work. All authors carried out laboratory tests. AHA wrote the manuscript, all authors revised and

approval the final version.

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