Hepatoprotective Effect of Pomegranate (Punica granatum L) Peels powder against Carbon Tetrachloride (CCl4)- Induced Hepatotoxicity in Rats

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Hepatoprotective Effect of Pomegranate (Punica granatum L) Peels powder against Carbon Tetrachloride (CCl4)- Induced Hepatotoxicity in Rats

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Abstract:

Background: Carbon tetrachloride (CCL4) is a colorless, volatile nonflammable liquid, a haloalkane and inhalation of its vapors can cause degeneration of the liver, kidneys and other organs through the generation of reactive oxidative stress. Pomegranate (Punica granatum L) peel, a plant by-product has been demonstrated to be a rich source of bioactive antioxidants mainly polyphenols which can inhibit and control hepatic injury with high efficacy and low toxicity.

Aim of work: This study aimed to investigate the hepatoprotective impact of pomegranate peel powder (POP) on the hepatic toxicity caused by carbon tetrachloride.

Methods: Male albino rats (n=30) were divided into 5 main groups (6 each). as follows: Group (1): Negative group control fed in basal diet, Group (2): used as the positive control group (+ve) and fed on basal diet. Groups (3, 4 and 5) fed on a basal diet with POP at levels of 2, 4 and 6 g/kg/diet/day, respectively. After, 29 days of the experiment, groups (2-5) were injected intraperitoneal with CCL4 (2 ml/b.w/rats to induce hepatic and other possible side effects as the renal disorders in rats, after 24 hours, rats were sacrificed and blood samples collected then centrifuged. Serum was kept frozen at - 20°C until analysis.

Results: Results of this study demonstrated that POP at all levels considerably improved the CCl4-induced changes in hepatic function parameters (ALP ,total protein, , ALT, albumin, GGT, total bilirubin, AST, and globulin) and renal functions (creatinine, urea nitrogen and uric acid). Furthermore, POP administrations significantly diminished the elevation of

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serum TC, TG, VLDL-C and LDL-C values and elevated serum HDL-Cvaluese. In addition, it restored the activities of antioxidant enzymes SOD and GSH which declined after CCl4 treatment and decreased MDA levels significantly. Conclusion: it could be concluded that pomegranate peel may guard against CCl4-induced hepatic injury through the organization of antioxidative activities.

Keywords: Liver injury, hepatic and renal functions, antioxidants, oxidative stress.

INTRODUCTION

Liver diseases are considered a major global health problem wherein the frontier reason of it is due to exposure to variant environmental pollutants, alcohol, chemicals (as carbon tetrachloride (CCl_4), xenobiotics, thioacetamide, paracetamol) and infections that produce reactive oxygen species (ROS), leading to tissue injury and hepatic damage **(Liu *et al.*, 2011 and Al-Sayed *et al.*, 2014)**.

Carbon tetrachloride (CCl_4) an industrial solvent, is widely used in lacquers, dry-cleaning industry, chemical laboratories to produce commercial fats, and other organic compounds, used as a refrigerant. Also, it is commonly as a typical toxic agent to induce oxidative stress and to scout the possible pathological mechanisms which contribute to the elaboration of hepatic injury in experimental models **(Noori *et al.*, 2009 ; Khan and Alzohairy, 2011; Jaeschke *et al* ., 2013 and Foaud *et al.*, 2018)**. CCl_4 converse through hepatic microsomal into free radical which initiate lipid peroxidation process leading to both pro-fibrotic and pro-inflammatory process **(Novo and Parola, 2012)**. It gives rise to hepatocyte injury which is characterized by centrilobular necrosis followed by liver fibrosis **(Abdel-Rahman and Abd El-Megeid, 2006)**. CCl_4 toxicity not only induced liver injury, but also other organs affected by its toxicity such as the kidney **(Foaud *et al.*, 2018** and **Ozturk *et al.*, 2012),** lung **(Ögetürk *et al.*, 2009)**, testis **(Abdel Moneim, 2016)** and brain **(Soliman and Fahmy, 2011).**

Several studies illustrated that antioxidants inhibit CCl_4 toxicity and hepatotoxicity, by elevating antioxidant enzyme activities including SOD,

catalase and GSH and preventing lipid peroxidation **(Foaud *et al.*, 2018 and Luo *et al.*, 2019 and Babagana *et al.*, 2020).** Pomegranate *(Punica granatum* L.*)* is one of the most popular medicinal plants belonging to the family Punicaceae **(Jurenka, 2008)**. Pomegranate possesses antioxidant, hydroxyl radical scavenging and anti-inflammatory activities **(Bachoual *et al.*, 2011; Abdel-Rahim *et al.*, 2013 and Altunkaya, 2014)**, anti-diabetic, anti-hypertensive, lipid-lowering **(Grabež *et al.*, 2020)** and anti-cancer characteristics **(Jeune *et al.*, 2005)**, hepato-protective effect **(Ashoush *et al.*, 2013; Bachoual *et al.*, 2011; Osman *et al.*, 2011** and **Wei *et al.*, 2015).**

Pomegranate peels have a rich variety of phenolic and polyphenol compounds mainly alkaloids, flavonoids (anthocyanins, flavons, flavonols, flavan-3-ols), tannins (ellagitannins such as, punicalagin, gallic acid, punicalin and ellagic acid) and organic acids (cumaric acid, chlorogenic acid, caffeic acid), that may be the responsible for its therapeutic properties (**Faria and Calhau, 2011).** In addition, it contains plenty of minerals and complex carbohydrates **(Noda *et al.*, 2002** and **Viuda-Martos *et al.*, 2010).**

This study aimed to investigate the hepatoprotective effect of pomegranate peel powder (POP) at different concentrations on oxidative stress and the toxicity induced by carbon tetrachloride (CCL_4).

Materials and methods

1- Materials:

1-1: Plants:

Punica granatum L. Pomegranate. peels were purchased as dried material from a spice dealer from the local market in Cairo.

1-2: Carbon tetrachloride (CCl_4):

Carbon tetrachloride (CCl_4) as a 10% liquid solution. Used as toxic material for hepatic poisoning in accordance with **Passmore & Eastwood (1986)**.

1-3: Rats & Diet:

Thirty mature male albino rats (Sprague - Dawley strain), their weight is 120±5 g. and their age is 8-12 weeks.

The basal diet elaborated by AIN-93 formulation **(Reeves *et al.*, 1993).** The Pomegranate powder plant was added at levels of 2%, 4% and 6% (on the diet).

2- Methods:

2.1. Elaboration of plant formulations:

Plant materials were grinded to produce a powder & retained in dim sealed glass bottles (in dry location) till the use time, in accordance with **Russo, (2001)** who declared that plant is best kept in dark & dry place to minimize oxidation of their components.

2.2. Analytical Methods

- Determination of moisture, Protein, fat, ash & fiber.
- Calculation of carbohydrates & energy value.

2.3. Experimental design:

Male albino rats (n=30) weighing 120±5 were housed in well-ventilated cages at 25 ± 3°C under hygienic conditions. Water and diet were available ad-libitum throughout 6 weeks of the investigation. The basal diet was elaborated in accordance with (NRC, 1995) and composed of corn oil (50 g/kg), casein (200 g/kg), cellulose (30 g/kg), DL-methionine (3 g/kg) , corn starch (497 g/kg), mineral mixture (100g /kg), vitamin mixture (20 g/kg) and sucrose (100 g/kg). Rats were allowed to acclimatize for a week before the experiment. After adaptation rats were divided into 5 main groups with 6 rats each. The groups divided as following: Group (1): Negative group control (fed in basal diet) Group (2): used as the positive control group (+ve) and fed on basal diet. Groups (3, 4 and 5) fed on a basal diet with pomegranate powder at (2, 4 and 4 g/kg/diet/day), respectively for 28 days. On day 29 from the beginning of the experiment, groups (2-5) were injected intraperitoneal with CCL4 (2 ml/b.w/rats) (previously diluted in liquid paraffin oil 1:1) to induce hepatic and other possible side effects as the renal disorders in rats, after 24 hours, sacrificed. At end of experiment period (29 days), rats were sacificed after fasting under

anesthesia. Blood samples collected, left to clot, and then centrifuged. Serum carefully aspirated and kept frozen till analysis time.

3-Biochemical analysis:

3.1. Estimation of hepatic functions:

Serum total protein, albumin, aspartate aminotransferases & alanine transaminase (AST & ALT), alkaline phosphates (ALP) & enzyme activity, serum globulin (G) value & serum total bilirubin were calculated.

3.2. Estimation of serum lipids:

Enzymatic colorimetric estimation of triglycerides, total cholesterol, VLDL and LDL were calculated.

3.3. Estimation of renal functions:

Serum creatinine, serum uric acid and urea in plasma were estimated.

3.4. Estimation of serum antioxidant parameters

superoxide dismutase (SOD) activity, glutathione (GSH), malondialdehyde (MDA) in accordance with **Nishikimi *et al.* (1972); Beuchamp and Fridovich (1971); Habig *et al.* (1974)** and **Ohkawa *et al.* (1979)** respectively.

4. Statistical analysis:

The resulted data statistically analyzed.

RESULTS AND DISCUSSION

Chemical composition of Pomegranate peel powder

The proximate chemical composition of the dessicated pomegranate peels is illustrated in Table (1). The fat, protein, ash, carbohydrates and fiber content of the pomegranate peel were 1.89, 2.32, 3.26, 6.75 and 85.78 D/W, respectively. It could be observed that pomegranate peel has a low content of protein and fat. These results agree with that observed **by Al Maghrabi (2003)** who reported that dried pomegranate peels contained 1.7% protein and 3.21 lipids.

Table (1): Chemical composition of pomegranate peel powder

Constitutes (%)	Value D/W
Protein	1.89
Fat	2.32
Ash	3.26
Fiber	6.75
Carbohydrates	85.78
Energy value (Kcal/100g)	371.56

D/W= Dry weight

Effect of Pomegranate peel treatment on the nutritional parameters of CCL4 intoxicated-rats

The changes in feed intake, feed efficiency ratio (FER) and body weight are illustrated in Table (1). The initial body weights of rats were similar in all groups and all of them gave positive body weight gain at the end of the experiment. Meanwhile, the CCL_4-treated group recorded the lowest body weight gain, feed intake and FER as compared with all groups these may be due to loss of appetite and the acute hepatic damage spotted in rats intoxicated using CCl_4. It was noticed that the treated rats with pomegranate peel powder (POP) at the levels of 2%, 4% and 6% showed a significant increase and protective effect in all nutritional parameters in comparison with the positive control group. On the other hand, POP at a level of 6% was the best protective ability against CCl_4 toxicity. These results are in accordance with **Hanaa (2014) and Nhung *et al.* (2014)** who stated that CCl_4 treatment groups were reduced the body weights of mice compared to that of the control group. **Abd El-Megeid & Abdel-Rahman (2006)** illustrated that administration of CCL_4 led to a decrease in weight gain of experimental rats compared to the negative control group, however, pomegranate peel revealed a highly noticeable improvement in rat's weight gain.

Table (2): Effect of Pomegranate peel treatment on the nutritional parameters of CCL4 intoxicated-rats

Parameters / Groups	Initial weight(g)	Final weight (g)	Weight gain (g)	Food intake (g/d)	FER
G(1): Normal control group (-ve)	125.32± 3. 35 a	237.08± 23.37 a	113.76± 8.11 a	15.94± 2.20 a	0. 255± 0.03a
G (2): CCL_4 control group (+ve)	122.24± 5.21 a	187.83± 19.34 b	66.59± 6.11 b	13.58± 2.03 b	0.175± 0.02 b
G (3): CCL_4 group+2% POP	121.32± 3.99 a	225.45 ± 24.12 a	105.13± 9.13 a	15.25± 2.32 a	0.246± 0.04 a
G (4): CCL_4 group+4% POP	125.91± 5.67 a	227.68± 20.84 a	102.77± 9.17 a	15.46± 2.21 a	0.237± 0.03 a
G (5): CCL_4 group+6% POP	125.22± 3.86 a	235.08± 22.08 a	109.86± 9.17 a	15.68± 2.92 a	0.250± 0.04 a

Mean values in each column having different superscripts (a, b) are significant.

Means with the same letter are insignificantly different.

Effect of Pomegranate powder treatment on serum liver parameters of CCL_4 intoxicated-rats

Data in Table (3) revealed that treatment with CCL_4 resulted in a considerable elevation in the activity of serum gamma-glutamyl transferase (GGT), total bilirubin, alanine transaminase (ALT), alkaline phosphatase (AlP) & aspartate transaminase (AST) compared to the negative control group. However, CCL_4 treatment showed a considerable declination in serum levels of albumin, total protein and globulin in comparison with the negative control group. It could be noticed that rats treated with pomegranate peel at all levels were showed a significant declination in the value of ALP, total bilirubin, ALT, GGT and AST in comparison to the positive control group. Regarding serum albumin levels, pomegranate peel

at all levels was showed a considerable elevation and it was observed no considerable variations between the negative control group and pomegranate peel groups at levels of 4% and 6%. Also, total protein and globulin showed a significant increase in comparison to the positive control group.

These results are in accordance with **Hsu *et al.* (2009)** & **Lee *et al.* (2007)**, it is known that chemical compounds as CCl_4 cause hepatic injury, that elevates ALT & AST in serum and this may indicate the index of hepatic injury. **Foaud *et al.* (2018)** revealed that CCL_4 administration caused a considerable elevation in serum ALT, AST and AlP, However, serum total protein showed no change. **Melo *et al.* (2015)** stated that rats exposed to hepatic injury by CCl_4 showed an elevation in ALT & AST levels as in comparison with the negative control. **El-Hadary and Hassanien (2016)** showed that the activities of ALP, ALT and AST enzymes in the CCl4-treated group were considerably elevated, in comparison with the negative control however, the protein parameters reduced in general (A/G ratio, globulin, albumin, and total protein). **Abdel-Rahman & Abd El-Megeid (2006)and Hanaa (2014)** reported that administration of CCl_4 resulted in liver damage monitored by blood plasma AlP, AST, bilirubin and ALT levels in mice however POP treatment showed significant amelioration to these changes . **Ali *et al.* (2021)** demonstrated that CCl4 intoxication induced a fundamental rise in the levels of serum ALP, ALT and AST in comparison with the normal control, on the other hand, pomegranate peel (PPE) and its fractions reduced these elevation in the serum level of liver enzymes to a salient extent, which is a clue to the repair of hepatocyte destruction and conservation of the cell membrane and hepatic architecture. **Zhai *et al.* (2018)** illustrated that the enzymic activities of AST, ALP and ALT were considerably raised in serum when mice were injected with 0.2 mL of CCl4 (0.2%), which suggested clear hepatotoxicity, on the contrary, these enzymic activities were considerably raised when mice administrated with polysaccharides isolated from pomegranate peel. Also, Punicalagin (PU) a bioactive antioxidant polyphenol found in pomegranates ameliorated the CCl4-induced increase of the serum AST, ALT, the activity of liver lactate dehydrogenase and the destruction of

histopathological structure and showed a hepatoprotective effect against CCl_4 **(Luo *et al.*, 2019)**. **Fakher El-Deen (2011)** illustrated that POP considerably declined the serum level of ALT and AST in high lipid diet-fed rats. **Toklu *et al.* (2007)** found that pomegranate peel extract decreased serum ALT and AST in rats with liver fibrosis. Pomegranate peel & seed extracts have been illustated to have protective impacts against CCl_4-induced hepatic fibrosis (**Wei *et al.*, 2015).** Also, **Abdel-Rahim *et al.* (2013)** showed that pomegranate seeds and peels produced significant improvement in the liver function (ALT, AST and bilirubin) of hypercholesterolemic rats.

Table (3): Effect of Pomegranate peel powder treatment on serum liver parameters of CCL4 intoxicated-rats

Parameters Groups	AST (Iu/l)	ALT (Iu/l)	ALP (Iu/l)	GGT U / L	Total bilirubin mg / dl	Albumin g / dl	T.protein g / dl	Globulin g / dl
G (1): Normal control group (-ve)	127.23 ±6.18 e	81.03 ±7.60 e	188.10 ±2.00 d	4.87 ±0.21 d	0.25 ±0.06 d	4.13 ±0.21 a	8.69 ±0.31 a	4.55 ±0.48 a
G (2): CCL4 control group (+ve)	180.87 ±6.53 a	217.60 ±15.95 a	285.27 ±15.28 a	7.8 ±0.82 a	1.86 ±0.15 a	2.33 ±0.42 c	5.20 ±0.36 d	2.87 ±0.67 c
G (3): CCL4 group+2% POP	166.47 ±8.80 b	174.83 ±5.08 b	230.17 ±1.76 b	6.87 ±0.45 b	1.08 ±0.14 b	2.93 ±0.25 b	6.03 ±0.21 c	3.10b ±0.26c
G (4): CCL4 group+4% POP	150.67 ±1.17 c	148.07 ±4.43 c	205.31 ±5.47 c	5.7 ±0.1 c	0.51 ±0.06 c	3.75 ±0.31 a	7.33 ±0.51 b	3.59 ±0.58 abc
G (5): CCL4 group+6% POP	139.43 ±4.07 d	115.43 ±4.33 d	191.22 ±7.13 cd	5.1 ±0.1 cd	0.31 ±0.03 d	3.93 ±0.11 a	7.90 ±0.36 b	3.97 ±0.47 ab

Mean values in each column having different superscripts (a, b, c, d, e) are significant.

Means with the same letter are non-significantly different.

AST: Aspartate transaminase ALT: Alanine transaminase ALP: alkaline phosphatase GGT: gamma-glutamyl transferase.

Effect of Pomegranate powder treatment on lipid profile of CCL_4 intoxicated-rats

Table (4) showed the changes in serum high-density lipoprotein-cholesterol (HDL-C), triglycerides (TG), very low-density lipoprotein-cholesterol (VLDL-C), total cholesterol level (TC) & low-density lipoprotein-cholesterol (LDL-C). It could be noticed that the serum levels of VLDL-C, TG, LDL-C and TC of positive control showed significant increases meanwhile serum level of HDL-C significantly decrease by CCL_4 administration. It was observed that POP treatment at different levels showed considerable declinations in serum VLDL-C, TG, LDL-C and TC values and a considerable raise in serum HDL-C value. However, they were still significantly higher than the negative control. G5 had a greater effect as a hypocholesterolemic agent.

These results are consistent with **El-Hadary and Hassanien (2016)** who revealed that treatment with CCl4 caused a considerable raise in all lipids parameters (total lipids, VLDL-C, TG, LDL-C and TC) except for HDL-C was increased. **Fakher El-Deen (2011)** demonstrated that pomegranate peel powder significantly decreases the serum level of LDLC, TC and TG whereas raised serum HDL-C in high lipid diet-fed rats. Also, administration of pomegranate peel powder or extract declined serum LDLC, TC and TG, and lipid peroxidation levels while HDL-C remained unchanged in obese rats fed on a hypercholesterolemic diet **Hossin (2009). Faria and Calhau (2011)** illustrated that pomegranate peel extract considerably declined serum TG and TC levels while increasing serum HDL-C levels in diabetic rats. Same results were recorded by **Sadeghipour *et al.* (2014)** who indicated that administration of pomegranate peel extract declined serum LDLC, TC, and TG whereas raised serum HDL-C in high lipid diet-fed male rats**.** Treatment with pomegranate seeds and desicatted peel induced an amelioration in the blood lipid profile of hypercholesterolemic rats **(Abdel-Rahim *et al.*, 2013** and **Salwe *et al.*, 2015). Haghighian *et al.* (2021)** illustrated that consumption of pomegranate peel extract considerably declined serum TG & TC levels in patients with osteoarthritis of the knee.

The lipid-declining effect of pomegranate has been attributed to its polyphenol components as hydrolyzed tannins & other components like dietary fiber and other carbohydrates **(Aviram *et al.*, 2008).** The pomegranate peel catechins improved serum blood lipid profile by preventing the key enzymes included in lipid biosynthesis & declined absorption of lipid by the intestine in obese subjects. Also, gallic acid, ellagic acid catechins and tannic acid have a free radical scavenging activity that cold inhibit peroxidation of lipid and improve lipid profile **(Suliburska *et al.* 2012;9 Sadeghipour *et al.*, 2014 and Grabež *et al.* 2020)** stated that treatment with pomegranate peel extract induced a considerable declining of plasma levels of low-density lipoprotein cholesterol / high-density lipoprotein cholesterol ratio (LDL-C / HDL-C), triglycerides, while the level of HDL-C.

Table (4): Effect of Pomegranate powder treatment on lipid profile of CCL_4 intoxicated-rats

Parameters Groups	**TC mg/dl**	**TG mg/dl**	**VLDL-c mg/dl**	**LDL-c mg/dl**	**HDL-c mg/dl**
G (1): Normal control group	82.27±4.16 d	68.13±2.97 d	13.63±0.59d	29.14±5.99e	39.50±2.29a
G (2): CCL_4 control group	128.57±4.71 a	107.33±10.66a	21.47±2.13a	78.87±3.55a	28.23±1.37c
G (3): CCL_4 group+2% POP	110.67±7.26 b	89.60±3.14 b	17.92±0.63b	61.58±5.76b	31.17±1.76b
G (4): CCL_4 group+4% POP	97.80±2.44 c	79.47±5.22bc	15.89±1.04bc	48.37±1.34c	33.53±3.02b
G (5): CCL_4 group+6% POP	92.27±2.89 c	73.27±1.42cd	14.65±0.28cd	39.91±3.66d	37.70±2.07a

Mean values in each column having different superscripts (a, b, c, d, e) are significant.
Means with the same letter are non-significantly different.
TC: Total cholesterol level. TG: Triglycerides. VLDL-C: Very low-density lipoprotein-cholesterol LDL-C: Low-density lipoprotein-cholesterol. HDL-C: high-density lipoprotein-cholesterol.

Effect of Pomegranate powder treatment on Kidneys function of CCL_4 intoxicated rats.

The effect of the three different levels of pomegranate peel powder on CCL_4-induced toxicity in rats is shown in Table (5). In normal control rats, the serum levels of urea nitrogen, creatinine & uric acid were 2.77±0.25, 20.1±1.23 and 0.41±0.02 mg/dl, respectively. However, in the positive group (+ve) serum uric acid urea nitrogen and creatinine levels were elevated significantly to 3.87±0.40, 50.87 and 0.71 mg/dl ($p < 0.05$), respectively. Rats treated with POP were showed a significantly decreasing value of uric acid, creatinine & urea in comparison with the positive control group. During the treatment period with the POP, especially group (5) treated with 6% POP renal function gradually improved, where there were no considerable differences between G (5) and negative control (Table 5).

These results are in harmony with **Fakher El-Deen (2011)** who stated that pomegranate peel powder significantly decreases the serum level of uric acid, creatinine and urea nitrogen in high lipid diet-fed rats. **Ahmed and Ali (2010)** found that administration with pomegranate peel extract results in significant decreases in serum levels of urea nitrogen and creatinine. **Abdel-Rahim *et al.* (2013)** showed that pomegranate seeds and peels produced considerable improvement in kidneys function (uric acid, urea and creatinine) of hypercholesterolemic rats.

Table (5): Effect of pomegranate peel powder treatment on Kidneys function of CCL_4 intoxicated rats.

Parameters / Groups	Uric acid mg/dl	Urea Nitrogen mg/dl	Creatinine mg/dl
G (1): Normal control group	2.77±0.25c	20.1±1.23d	0.41±0.02c
G (2): CCL4 control group	3.87±0.40a	50.87±3.05a	0.71±0.03a
G (3): CCL4 group+2% POP	3.42±0.31ab	40.53±3.99b	0.66±0.03a
G (4): CCL4 group+4% POP	3.07±0.21bc	31.17±3.40c	0.51±0.02b
G (5): CCL4 group+6% POP	2.83±0.25c	24.83±4.03d	0.46±0.05bc

Mean values in each column having different superscripts (a, b, c, d) are significant. Means with the same letter are non-significantly different.

Effect of Pomegranate powder treatment on antioxidant parameters of control and CCL_4 treated rats

From the presented data in Table (6) it could be noticed that CCL_4 administration caused a demotion in superoxide dismutase (SOD) and glutathione reductase (GSH) levels, however, it showed a raise of malondialdehyde (MDA) levels in comparison with the negative control group (-ve). It's clear that Treatment with POP at all levels markedly reversed the alterations in biochemical parameters induced by CCL_4. G (5) which was treated with 6% POP had the best result in increasing SOD and GSH levels and lowering the elevation of MDA levels caused by the administration of CCL_4.

These results are comparable with **Cai *et al.* (2015); Foaud *et al.* (2018) and El-Hadary and Hassanien (2016)** who revealed that CCL_4 administration caused a considerable raise in MDA content in the hepatic tissue which is a reflection of the lipid peroxidation occurrence result in hepatic tissue destruction and antioxidant defense mechanisms failure **(Termini, 2000).** On the other hand, the GSH and SOD level was considerably declined in CCl4-treated rats **(Hadary and Hassanien, 2016)**. **Ali *et al.* (2021)** demonstrated that CCl4 intoxication considerably raised the MDA content while declining SOD & GSH levels in the hepatic tissues, in comparison with the normal control group indicating severe oxidative stress occurrence, In contrast, treatment with PPE and its fractions significantly attenuated this. PPE was illustrated as the richest origin of antioxidants among peel extracts of the most consumed fruits **(Okonogi *et al.*, 2007** and **Parmar and Kar, 2007)**. It is demonstrated to be higher antioxidant activity in comparison with pomegranate seed and lea extracts **(Tehranifar *et al.*, 2011).** Alternatively, **Haghighian *et al.* (2021)** reported that consuming pomegranate peel powder significantly decreased serum levels of MDA and significantly increased SOD, GPx, and TAC compared to the control group. Also, pomegranate peel extracts improved SOD activity & attenuated lipid peroxidation in CCl_4-induced hepatic destruction **(Murthy *et al.*, 2002).** It declined MDA and raised SOD, catalase (CAT) & GSH activities on rats' brains **(Moneim, 2012). Waly *et al.* (2012)** indicated that pomegranate peel

extract improved the impairment SOD, GPX and TAC. Moreover, pomegranate peel was reported to reduce lipid peroxidation & increase CAT and SOD levels in rats with hyperlipidemia **(Sadeghipour *et al.,* 2014)**. Such antioxidant ability and reduction of oxidative stress mediators of pomegranate have been attributed to its polyphenolic contents for its free radical scavenging properties(**Moghaddam *et al.,* 2013; Matthaiou *et al.,* 2014 & Husain *et al.,* 2018)** Punicalagin diminished oxidative stress by declining the hepatic MDA level and raising the activities of hepatic SOD and GSH **(Luo *et al.,* 2019)**. SOD act as a complicated system of defense against reactive oxygen intermediates, it neutralizes the superoxide anion which has a substantial role in inflammation **(Afonso *et al.,* 2007)**. Also, **Mahdavi and Javadivala (2021)** stated the convenient properties of pomegranate in ameliorating clinical manifestations, diminishing inflammatory and oxidative stress. Moreover, **Zhai *et al.* (2018)** revealed that CCl_4 decreased enzymic activities of GSH-Px, CAT, T-SOD & non-enzymic activity of GSH, suggesting that CCl4-induced oxidative hepatic injury had been set up, these activities were significantly raised when mice administrated with polysaccharides isolated from pomegranate peel, suggesting that the polysaccharides from pomegranate peel had considerable hepatoprotective & antioxidant capability against CCl4-induced oxidative destruction in mice,

Table (6): Effect of Pomegranate powder treatment on antioxidant parameters of control and CCL4 treated rats

Parameters / Groups	SOD U/g protein	GSH U/mg protein	MDA µmol/mg protein
G (1): Normal control group	210.67±11.02a	142.21±1.06a	4.64±0.39e
G (2): CCL_4 control group	108.33±9.02d	53.32±0.76d	11.17±0.38a
G (3): CCL_4 group +2% POP	140.17±5.10c	82.22±0.60c	9.10±0.21 b
G (4): CCL_4 group +4% POP	170.63±7.07b	92.42±0.70c	7.50±0.40 c
G (5): CCL_4 group +6% POP	197.67±7.51a	121.43±0.50b	5.53±0.41d

Mean values in each column having different superscripts (a, b, c, d, e) are significant.

Means with the same letter are non-significantly different.

SOD: Superoxide dismutase. GSH: Glutathione reductase. MDA: Malondialdehyde (MDA).

Conclusion

Pomegranate (*Punica granatum* L) peel is a rich source of health-promoting antioxidants and possessed a hepatoprotective effect against CCl4-induced toxicity in rats.

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التأثير الواقي لمسحوق قشور الرمان ضد السمية الكبدية المستحدثة برابع كلوريد الكربون (CCl4) في الفئران

الملخص العربي:

يعتبر رابع كلوريد الكربون سائل عديم اللون متطاير و غير قابل للاشتعال، ويمكن أن يتسبب استنشاق أبخرته في تلف الكبد والكلى وبعض الأعضاء الأخرى من خلال توليد الإجهاد التأكسدي. هذا ومن جانب اخريعتبر قشر الرمان، منتج ثانوي غني بمضادات الأكسدة النشطة بيولوجيًا يحتوى بشكل رئيسي على البوليفينولات الذي يمكن ان تقى من تلف الكبد بفعالية كبيرة وبنسبة سميةمنخفضة. و تهدف هذه الدراسة إلى التحقق من التأثير الوقائي للكبد لمسحوق قشر الرمان على سمية الكبد المستحدثة برابع كلوريد الكربون.حيث تم تقسيم ذكور الفئران البيضاء (العدد = ٣٠) إلى ٥ مجموعات رئيسية (٦ فءران لكل مجموعة). كالتالي: المجموعة (١): المجموعةالضابطة غذيت على النظام الغذائي الأساسي، المجموعة (٢): تستخدم كمجموعة ضابطة موجبة (+ ve) وتغذت على النظام الغذائي الأساسي. المجموعات (٣ و ٤ و ٥) تتغذى على نظام غذائي أساسي يحتوي على مسحوق قشور الرمان بنسب ٢ و ٤ و ٦ جم / كجم / وجبة/ يوم، على التوالي. و بعد ٢٩ يومًا من التجربة، تم حقن المجموعات (٢- ٥) باستخدام (CCL4٢مل /كجم من وزن الجسم / الجرذان) لاصابةالكبد واحداث الآثار الجانبية المحتملة الأخرى مثل الاضطرابات الكلوية في الجرذان ، وبعد ٢٤ ساعة، تم ذبح الفءران وتجميع الدم. تم تجميع مصل الدم بعد اجراء عمليةالطرد المركزى ثم تم حفظ المصل مجمدا عند - ٢٠ درجة مئوية حتى اجراءالتحاليل. لقدأظهرت نتائج هذه الدراسة أن مسحوق قشور الرمان بجميع النسب المستخدمة حسنت بشكل ملحوظ التغيرات التي سببها CCL4 في وظائف الكبد (AST، ALT، ALP، GGT ، البيليروبين والألبومين والبروتين الكلي والجلوبيولين) ووظائف الكلى (الكرياتينين واليوريا وحمض البوليك). علاوة على ذلك، قللت قشور الرمان بشكل كبير من ارتفاع قيم TC و TG و VLDL-C و LDL-C في الدم مع زيادة مستوى الـ HDL في الدم. بالإضافة إلى ذلك، أعاد نشاط إنزيمات مضادات الأكسدة SOD و GSH التي انخفضت بعد المعالجةبـ CCl4 وخفضت مستويات MDA بشكل ملحوظ. ويمكن الاستنتاج أن قشر الرمان قد يقي من السمية الكبدية المستحدثة برابع كلوريد الكربون (CCl4) في الفءران من خلال تنظيم الأنشطة المضادة للأكسدة.

الكلمات المفتاحية: تلف الكبد، وظائف الكبد والكلى، مضادات الأكسدة، الإجهاد التأكسدي.