# Protective Role of Pomegranate Peel Extract on Testis in Adult Male Rabbits Treated with Carbon Tetrachloride

**Wassan M. Hussen and Jawad K.Arrack**
Department of Physiology and Pharmacology, College of Veterinary Medicine, Baghdad University, Iraq
E-mail: wassannet2000@yahoo.com
Accepted on:30/10/2013

## Summary

The aim of the present study is to prepare ethanol extract of Pomegranate peel and the effects of this extract on testicular weight to body weight ratio, Serum cholesterol, testosterone concentration and histopathological changes of testes in rabbits treated with carbon tetrachloride ($CCl_4$). Twenty four adult male rabbits were used. They were divided randomly into four equal groups. Animals were treated for 56 days as following: Rabbits of the 1st group were received 1 ml distal water orally once a day and olive oil 0.5 ml /kg B.W. I.P twice a week as control group. The second group were treated I.P with 500mg / kg B.W. of $CCl_4$ mixing with equal volume of olive oil (0.5 ml/kg B.W.) twice a week (group T1). The third group was received pomegranate peel extract orally (100 mg/kg B.W) once a day and olive oil 0.5 ml /kg B.W. I.P twice a week (group T2). The fourth group were received pomegranate peel extract (100 mg/kg B.W) once a day oral I.P with 500 mg / kg B.W. of $CCl_4$ mixing with equal volume of olive oil (o.5 ml/kg B.W.) twice a week (group T3). Blood samples were collected at (0, 14, 28, 42 and 56) days for measuring testosterone concentration, Serum cholesterol after treatments. Animals weighed and scarified and testis were removed and weighed, Samples of testis were taken for histopathological study. The results of the present study showed that treatment with pomegranate peel extract causes a significant ($P<0.05$) increase in testicular weight to body weight ratio. Also a significant ($P<0.05$) decreased of serum cholesterol and a significantly ($P<0.05$) elevation of testosterone concentration were observed. Histopathological examination of the testis was revealed that the extract of Pomegranate peel protect the testis against lesions caused by $CCl_4$. In conclusion, Pomegranate peel extract could protect the tissue of testicles from $CCl_4$ perhaps, by its anti-oxidative effect of pomegranate peel extract, hence eliminating the deleterious effects or toxic effect of $CCl_4$.

**Keywords: Pomegranate peel extract, Testis rabbits, $CCl_4$.**

---

## Introduction

Pomegranate (*Punicagranatum L.*) has been used in the folk medicine of many cultures especially in the Middle East (1). Edible parts of pomegranate fruit represent 52% of total fruit weight, comprising 78% juice and 22% seed (2). Non edible part called pomegranate peel extract or husk extract is primarily composed of alkaloids and polyphenols, which is composed from Anthocyanidins, Pelargonidin, Ellagotannins, Gallic acid, Ellagicacd, Psuedopelletierine and Isopelletierine (3 and 4). Pomegranate has become more popular because of the attribution of important physiological properties, such as anticancer (5 and 6) anti-proliferative apoptotic (7) cardio protective (8) anti-hyperlipidemic (9). Dried fruit peel is used for diarrhea and to treat respiratory and urinary tract infections. Also, pomegranate fruit peel exerted diverse pharmacological functions as antioxidant activit, antifertility effect, cytotoxic activity, hepatoprotective activity and hypoglycemic activity (10-15). Additionally, many investigators (16 and 17) have reported that pomegranate and its derivatives have free radical scavenger and potent antioxidant activity.

Carbon tetrachloride ($CCl_4$) is a halo alkane used in a variety of industrial and chemical applications. It has been widely used for its solvent properties, as intermediate in the synthesis of chlorofluorocarbons (18). $CCl_4$ leads to generation of free radicals caused cell injury and apoptosis to cells (19). It is known to be hepatotoxic as well as nephrotoxic to experimental animals (20). It also causes a decrease in the amount of mature spermatozoa in testis (21) with degeneration in sperm and

loss of sperm in epididymis tube in experimental animal (22).

## Materials and Methods

Carbon tetrachloride ($CCl_4$) was obtained from the chemistry department / Baghdad University. While Pomegranate peel purchased from local market was dried and powdered before extraction.

A hundred gm of pomegranate peel dry powder was taken, mixed with 50% ethyl alcohol and heated to (60 -70) C̊ for two hours by using Soxhelt extractor then separated by centrifuge 5000 rpm for 20 minutes. The supernatant solution was collected in sterile container, this process was returned three times then the solution was collected in sterile container (23). After the above process done the ethyl-alcohol was removed from the solution by Rotary Evaporator, then the final result of the extracted material was kept, the above extracted material was alcoholic extract of pomegranate (24).

Twenty four adult male rabbit were obtained from local market of Baghdad, housed in cages and placed in room for two weeks for adaptation. Room temperature was maintained at (21 - 25˚C), air of the room was changed continuously by using ventilation vacuum and with light/dark cycle of 12:12 h/day. Animals were housed at the animal house, College of Veterinary Medicine, Baghdad University and were fed on pellet of freshly prepared ration along the experimental periods.

The rabbits were divided randomly into four equal groups and handled as follows: Control Group: Rabbits treated orally with distal water daily via Gavage needle and olive oil (0.5ml /Kg B.W. I.P) twice a week for 56 days. Group T1: Rabbits treated with (0.5 ml /Kg B.W. I.P) of $CCl_4$ and mixed with an equal volume of olive oil (0.5 ml / Kg B.W.) twice a week for 56 days (25). Group T2: Rabbits treated orally with 100 mg /Kg B.W. of Pomegranate Peel Extract (PPE) daily via Gavage needle and with olive oil (0.5 ml /Kg B.W. I.P) twice a week for 56 days (26). Group T3: Rabbits treated orally with 100 mg /Kg B.W. of PPE daily via Gavage needle and (0.5 ml /Kg B.W. I.P of $CCl_4$ mixed with an equal volume of olive oil (0.5 ml / Kg B.W.) twice a week for 56 days.

Blood sample were collected at 0, 14, 28, 42 and 56 days of experiment via cardiac puncture technique. Blood collected in vacuum test tubes (for serum separation). Serum separated from coagulated blood sample by centrifugation at 2500 rpm for 15 min. and kept by freezing at -20 C̊ until used. Cholesterol in the sample was measured by spectrophotometer. Measure the absorbance (A) of the Standard and sample at 500 nm against the blank (27). Testosterone hormone concentration measured by ELISA method according to Kit manufacturer's instructions.

After the end of experiment period animals weighted by electrical balance and then sampling. Testes were obtained and weighted by sensitive balance after being cleaned from the accessory connective and adipose tissues. The testes were excised and preserved in 10% formalin buffer solution until preparation of histopathological section. Tissue was cut at 3-5µm and embedded in paraffin and several tissue sections of testes was stained with hematoxylin-Eiosin stain (H&E) for histopathological study (28).

Results are expressed as mean $\pm$ SE. Statistical analysis of data was performed on the basis of Chi square (x2), two-way analysis of variance (ANOVA II), and one- way analysis of variance. Group differences were determined using least significant difference (LSD) test at $P<0.05$ (29).

## Results and Discussion

Table (1) showed a significant decrease of testosterone concentration in T1 group as compared with the control and T2 group at along time of the experimental periods. Also within group showed a significant decrement of testosterone in T1 group as compared with pretreated time. There were no significant differences in testosterone concentration in T2 and T3 group as compared with the control group, whereas, T3 group showed a significant increase in testosterone concentration as compared with T1 group. Also in T3 group within time showed significant decrease of testosterone as compared with pretreated time.

$CCl_4$ caused advance cirrhosis which may lead to testicular atrophy and low serum testosterone (30). Also hypogonadism result from oxidative stress by $CCl_4$ (31 and 32) lead to decrease in leydig cell number which is responsible for synthesis and secretion of testosterone hormone (33). $CCl_4$ toxicity affected the pituitary gland causing a reduction in level of FSH and LH leading to inhibition of steroid biosynthesis by leydig cells which may cause decrease of testosterone (34).

The elevation of hormone level in T3 group could be due to pomegranate which contains a group of phytochemicals called ellagitannins. Several of these, including ellagic acid and gallagic acid, which inhibit aromatase and 17 beta-hydroxysteroid dehydrogenase enzymes or have anti estrogenic activity (35 and 36), these prevent conversion of testosterone to estrone and elevation of testosterone concentration. Perhaps presence of ellagic acid , ellagitanin, gallic acid, flavonoid, punicalgina, anthocyanin and hydrolysable tannin found in PPE (4) cause elevation of testosterone by protect leydig cell and spermatozoa from toxicity induced by some chemical agent (37). (Table, 2) showed that testicular weight to body weight ratio significant increased in T2 group as compared with the control, T1 and T3 groups.

The results also showed testicular weight to body weight ratio significant increased in (T1group) as compared with the control group. While showed a significant decreased when compared with T2 group. Data in (Table, 2) also demonstrated that there were no significant differences in T3 group when compared with control group, whereas, showed significant elevation as compared with T1 group.

A decrease in testicular weight to body weight ratio in T1 group is most likely due to a decrease in the level of serum testosterone (38). Furthermore, concentrations of testosterone play an important role in spermatogenesis (39), so a significant decrease of testosterone may cause a decrease in the number and function of somatic and germinal cells of testis followed by a testicular weight reduction.

Some experimental studies demonstrated that $CCl_4$ lead to generation of free radicals and produced cell injury (19 and 40) and also exposure of animal to $CCl_4$ causes advance cirrhotic which lead to testicular atrophy and gonadal dysfunction (31). Animals in (T3 group) showed a significant increase in testicular weight to body weight ratio as compared with T1 group, that may be due to effect of PPE compound as antioxidant which remove oxidative stress and free radicals (41) generated by $CCl_4$ administration.PPE provided marked increases in all the spermatogenic cells ranging from spermatogonia, spermatocytes, spermatids to spermatozoa as compared to the control. Additionally PPE provided an increase in the diameter of seminiferous tubule and germinal cell layer thickness (42).

**Table ,1: Effect of $CCl_4$, (PPE) and (PPE+ $CCl_4$) on testosterone hormone concentration (ng/ml) in adult male rabbit. M ± S.E. (n = 6).**

| Group / Time | Control group | T1 Group ($CCl_4$) | T2 Group (PPE) | T3 Group ($CCl_4$+PPE) |
|---|---|---|---|---|
| Zero time | 4.18 ± 0.65 A a | 4.52 ± 0.71 A a | 3.88 ± 1.42 A a | 4.23 ± 0.92 A a |
| 14 day | 3.95 ± 0.75 A a | 1.13 ± 0.18 B b | 3.36 ± 0.97 A a | 2.87 ± 0.71 B ab |
| 28 day | 4.45 ± 0.47 A a | 1.23 ± 0.32 B b | 4.51 ± 0.44 A a | 2.81 ± 0.55 B ab |
| 42 day | 3.99 ± 0.73 A a | 1.32 ± 0.41 B b | 4.44 ± 0.46 A a | 2.47 ± 0.20 B ab |
| 56 day | 4.29 ± 0.58 A a | 1.32 ± 0.24 B b | 5.10 ± 0.39 A a | 2.07 ± 0.44 B ab |

L SD =1.7

**- T1: Animals received $CCl_4$ 500mg/kg B.W. - T2: Animals received PPE 100 mg/kg B.W. -T3 Animal received PPE 100mg/kg and $CCl_4$ 500 mg/kg - Capital letters denote significant differences within group (P<0.05). -Small letters denote significant differences between groups (P<0.05).**

**Table , 2: Effect of $CCl_4$, (PPE) and (PPE+ $CCl_4$) on testicular weight to body weight ratio (%) in adult male rabbit.**

| Group / Time | Control group | T1 Group ($CCl_4$) | T2 Group (PPE) | T3 Group ($CCl_4$+PPE) |
|---|---|---|---|---|
| After 56 day of treatment | 0.185 ±0.003 b | 0.143 ± 0.005 c | 0.210± 0.007 a | 0.177 ± 0.005 b |

**-**

**T1: Animals received $CCl_4$ 500mg/kg B.W. - T2: Animals received PPE 100 mg/kg B.W.**
**-T3 Animal received PPE 100mg/kg and $CCl_4$ 500 mg/kg.**
**- Small letters denote significant differences between groups (P<0.05).**

The result of (Table, 3) showed a significant increase in the value of serum cholesterol in (T1 group) as compared with the control group and other groups at days 14, 28, 48 and 56 of experimental periods. Animals in T2 group showed a significant decrease in the values of serum cholesterol at a day 28, 42 and 56 of experiment as compared with control group and other treated groups. Moreover, T3 group showed significant increase in the values of total serum cholesterol as compared with control group at experimental periods, whereas, significant decrease in the values of serum cholesterol in (T3 group) as compared with T1 group.

There was a significant increase in the value of serum cholesterol in T3 group at day 14, 28, and 42 of experiment compared with zero time. While group (T1) showed significant increase in the value of total serum cholesterol as compared with zero time (Table, 3).

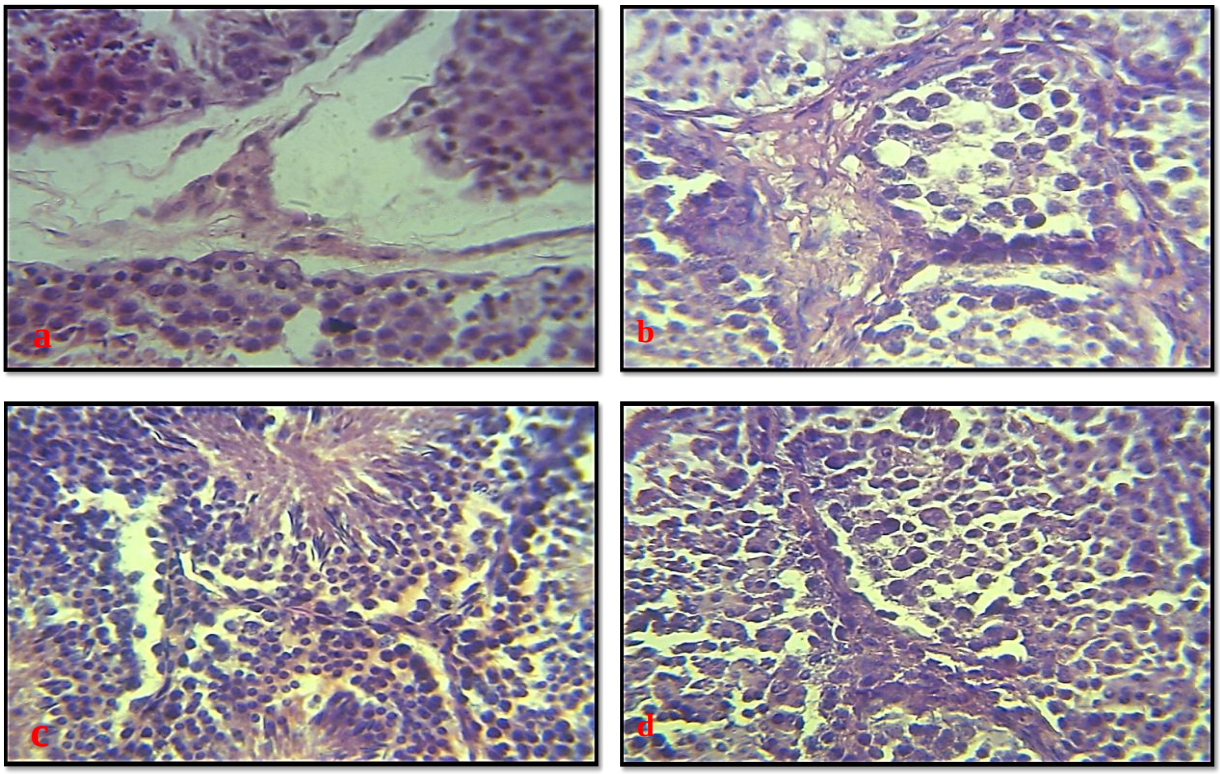
Among various chemical agent, $CCl_4$ has been thoroughly stated for its hepatotoxic properties (43) which in turn increase the accumulation fat in the liver lead to hyperlipidemia(44). The increase in serum cholesterol value in $CCl_4$ treated animals was attributable mainly an increase in cholesterol in LDL (Low-density lipoprotein) fraction and decrease in HDL (High-density lipoprotein) (44). Serum LDL cholesterol levels are known to be regulated by receptor mediated clearance of the lipoprotein, so the increase of serum LDL in $CCl_4$ induced animals cause reduction of catabolic pathways. HDL inhabit the uptake of LDL by the arterial wall facilitates the transport of cholesterol from peripheral tissue to liver where it's catabolized excreted from the body (45). Polyphenol substance in pomegranate causes decreased cholesterol absorption, increase fecal excretion of cholesterol, had a beneficial effect on enzymes involved in cholesterol metabolism, significantly reduced total and LDL cholesterol, and improved total /HDL and LDL/HDL cholesterol ratios (46). The restore level of total serum cholesterol in T3 group may be due to a powerful antioxidant capacity of ellagic acid present in PPE and effective scavenger of several reactive oxygen species (47). Also may be due to PPE contain water soluble polyphenols, anthocyanins and hydrolysable tannin (48) work as antioxidant compound and play important role in reduce liver toxicity and return it to normal function. Also, the greatest mechanism of polyphenol found in PPE for combating cholesterol (26).

**Table , 3: Effect of CCl4, (PPE) and (PPE+ CCl4) on total serum cholesterol (mg / dl ) in adult male rabbits. M ± S.E. (n = 6).**

| Group / Time | Control group | T1 Group ($CCl_4$) | T2 Group (PPE) | T3 Group ($CCl_4$+PPE) |
|---|---|---|---|---|
| Zero time | 41.16 ±2.26 A a | 46.33 ± 3.00 C a | 42.66 ± 0.95 A a | 48.50 ± 4.16 C a |
| 14 day | 48.33 ± 5.14 A c | 121.50 ±7.56 A a | 41.16 ± 1.25 A c | 80.33 ± 5.26 A b |
| 28 day | 44.166± 3.45 A c | 114.00 ± 5.38 A a | 32.66 ± 3.29 AB c | 85.33 ± 6.52 A b |
| 42 day | 41.00 ± 3.65 A c | 117.00 ± 6.81 A a | 27.66 ± 2 .18 B d | 83.66 ± 5.22 A b |
| 56 day | 39.33 ± 2.40 A c | 101.66 ± 2.90 B a | 23.83 ± 2.19 B d | 71.66 ± 5.94 B b |

**L SD =11.45**

**- T1: Animals received CCl4 500mg/kg B.W.- T2: Animals received PPE 100 mg/kg B.W.-T3 Animal received PPE 100mg/kg and CCl4 500 mg/kg. - Capital letters denote significant differences within group (P<0.05).-Small letters denote significant differences between groups (P<0.05).**




**Figure, 1: Histopathological examination of testis rabbits (a) control group. (b) T1 group treated with 500mg/kg mixed with 0.5 ml of olive oil.(I.P) Twice daily. (c)T2 group treated with 100mg/kg PPE daily with 0.5 ml of olive oil.(I.P) Twice daily (d) T3 group treated with 100mg/kg PPE plus 500mg/kg $CCl_4$ mixed with 0.5 ml of olive oil(I.P) Twice daily .After 56 day of treated.**

Histopathological examination of testis rabbits in T1 group (fig. 1-b) showed fibrosis of interstitial tissue which replace the atrophy leydig cell, reduction in size of the seminiferous tubule, degeneration and vacuolation in spermatogonia, spermatocytes and less number of germ cells, irregular seminiferous tubules and reduced seminiferous epithelial layers. Atrophy of leydig cell may be due to $CCl_4$ induce oxidative stress and increase free radical in testis therefore may cause destruction to the leydig cell and atrophied of leydig cells (32).

Whereas, testis rabbits in T2 group showed full differentiation and proliferation of spermatogensis in the center of seminiferous

tubules were compact with sperm, and protein material in the lumen of seminiferous tubule (fig. 1-c), This may be due to an increase of testosterone concentration which supports spermatogenesis and increases spermatocyte maturation; facilitating round to elongated spermatid progression and the release of spermatids from Sertoli cells may cause differentiation and proliferation of spermatogenesis (49 and 50). Section of testis tissue obtained from rabbits in T3 group showed a moderate proliferation and differentiation of spermatogenesis, proliferation of leydig cells and all stage of spermatogenesis (fig. 1-d) as well as epididymis lumen filled with sperm cell. This results may be due to that pomegranate has a protective role against $CCl_4$ toxicity (contain ellagic acid , ellagitanin(51), gallic acid, punicalgina and anthocyanin are also found in the hydrolysable tannin (4), therefore moderate proliferation and differentiation of spermatogenesis.

Conclusion from these results, pomegranate peel protect the testis from $CCl_4$ damages perhaps, by its anti-oxidative effect, hence eliminating the deleterious effects of toxic metabolites from $CCl_4$.

## References


1. Gurib-Fakim, A. (2006). Medicinal plants: traditions of yesterday and drugs of tomorrow. Mol. Aspects Med., 27:1- 93.
2. El-Nemr, S.E.; Ismail, I.A. and Ragab, M. (1990). Chemical composition of juice and seeds of pomegranate fruit. Nahrung., 34:601e6
3. Zakir, R. (2005). Pomegranate Extract and determination of content. J. Nutr. Biochem., 55:180-120.
4. Navindra, P.S.; Susanne, M.; Henninga, Y.Z.; Marc, S. and Daved, H. (2006). Pomegranate juice ellagitanin metabolites are present in human plasma and some persist in urine for up to 48 houre. J. Nutr., 136:2481-2485.
5. Afaq, F.; Saleem, M.; Krueger, C.G.; Reed, J.D. and Mukhtar, H. (2005). Anthocyanin and hydrolyzable tannin-rich pomegranate fruit extract modulates MAPK and NF-kB pathways and inhibits skin tumorigenesis in CD-1 mice. J. Int. Cancer., 113:423-33.
6. Lansky, E.P.; Harrison, G.; Froom, P. and Jiang, W.G. (2005). Pomegranate (Punicagranatum) pure chemicals show possible synergistic inhibition of human PC-3 prostate cancer cell invasion across Matrigel. Invest New Drugs., 23:121.
7. Seeram, N.P.; Adams, L.S.; Henning, S.M.; Niu, Y.; Zhang, Y.; Nair, M.G. and Heber, D. (2005). In vitro anti-proliferative, apoptotic and antioxidant activities of punicalagin, ellagic acid and a total pomegranate tannin extract are enhanced in combination with other polyphenols as found in pomegranate juice. J. Nutr. Biochem., 16(6): 360-367.
8. Sumner, M.D.; Elliott-Eller, M. and Weidner, G. (2005). Effects of pomegranate juice consumption on myocardial perfusion in patients with coronary heart diseases. Am. J. Cardiol., 96:810.
9. Fuhrman, B.; Volkova, N. and Aviram, M. (2005). Pomegranate juice inhibits oxidized LDL uptake and cholesterol biosynthesis in macrophages. J. Nutr. Biochem., 16:570.
10. Thring, T.S.; Hili, P. and Naughton, D.P. (2009). Anti-collagenase,anti-elastase and anti-oxidant activities ofextracts from 21 plants. BMC Complementary and Alternative Medicine, 9: 17-27.
11. Gujraj, M.L.; Varma, D.R. and Sareen, K.N. (1960). Oralcontraceptives. Part 1. Preliminary observationson the antifertility effects of some indigenousdrugs. Indian J. Med. Res., 48: 46-51.
12. Sato, A. (1990). Cancer chemotherapy with orientalmedicine. 1. Antitumor activity of crude drugswith human tissue cultures, in In vitroscreening. Int. J. Orient. Med., 15 (4): 171-183.
13. Kulkarni, A.P.; Mahal, H.S.; Kapoor, S. and Aradhya, S.M. (2007). In vitro studies on the binding, antioxidant, and cytotoxic actions of punicalagin. J. Agric. Food Chem., 55(4):1491-1500.
14. Murthy, K.N.; Jayaprakasha, G.K. and Singh, R.P. (2002). Studies on antioxidant activities of pomegranate peel extract using in vivo models. J. Agri. Food Chem., 50: 4791-4795.
15. Hontecillas, R.; O'Shea, M.; Einerhand, A.; Diguardo, M. and Bassaganya-Riera, J.

(2009). Activation of PPAR gamma and alpha by punicic acid ameliorates glucose tolerance and suppresses obesity-relatedinflammation. J. Am. Coll. Nutr., 28(2):184-95.

16. DeNigris, F.; Williams-Ignarro, S. and Lerman, L.O. (2005). Beneficial effects of pomegranate juice on oxidation-sensitive genes and endothelial nitric oxide synthase activity at sites of perturbed shear stress. Proc. Natl. Acad. Sci. U. S. A., 102:4896-4901.
17. Rosenblat, M.; Hayek, T. and Aviram, M. (2006). Anti-oxidative effects of pomegranate juice (PJ) consumption by diabetic patients on serum and on macrophages. Atherosclerosis.,187:363e71
18. ATSDR (Agency for Toxic Substance and Diseases Registry) (1994). Toxicological profile for carbon tetrachloride, U.S. Department of Health and Human Services, Public Health Service, TP-39/02. Atlanta, CA.
19. Kamel, H.H.; Azza, H. A.; Walaa, M.S. A. and Amira, H. M. (2010). Protective Effect of some Antioxidants against $CCl_4$-Induced Toxicity in Liver Cells from BRL3A Cell Line. J. Amer. Sci., 6(10).992 -1003.
20. Karadeniz, A.; Yildirim , A.; Karakoc, A.; Kalkan, Y. and Celebi, F. (2009). Protective effect of Panax ginseng on carbon tetrachloride induced liver, heart and kidney injury in rats. Revue Méd. Vét., 160(5):237-243.
21. Manjrekar, A. P.; Jisha, V.; Bag, P. P. ;Adhikary, B.; Pai, M. M.; Hegde, A. and Nandini, M. (2008). Effect of Phyllanthusniruri Linn. treatment on liver, kidney and testes in $CCl_4$ induced hepatotoxic rats. Indian J. Exp. Biol., 46:514-520.
22. deLamirande, E.; Leclerc, P. and Gagnon, C. (1997). Capacitation as a regulatory event that primes spermatozoa for acrosome reaction and fertilization. Mol .Hum. Report, 3:175-94.
23. Mohamed, B.M. (2010). Study the protective role of ellagic acid extracted from pomegranate peel (*Punicagranatum L.*) on Liver and Kidney function in rats treated with lead acetate. MS.C. thesis, collage of Veterinary Medicine / University of Baghdad., 47-71.
24. Godfred,A. (2004). Analysis of plant polyphenols by high performance liquid chromatography /Mass Spectrometry and protein binding .M.S.c. Thesis Miami University.
25. Yim, Y.; Lee, H.; Hong, K; Kim, Y.; Lee, B.; Kim, T. and Yi, J. (2006). Hepatoprotective effect of manual acupuncture at acupoint GB34 against $CCl_4$-induced chronic liver damage in rats. World J. Gastroenterol .,12(14): 2245-2249.
26. AL-Muslehi, M.S. (2010). Protective Role of Ethanolic extract of Pomegranate (Punicagranatum L.) on certain physiological aspects of Adrenal , Pancreas and Blood rats toxicated with Lead Acetate. MS.c. thesis, Collage of Veterinary Medicine / University of Baghdad., p:33.
27. Ellefson, R. and Garaway, W. (1976). Lipid and lipoproteins In: undamentals of clinical chemistry. Tietz, NW (Ed)., Pp:512-514.
28. Coles, E.H. (1974).Veterinary Clinical Pathology 2nd Ed. Saunders company. Philadelphia, London, Toronto. Chapter 3 and 4., Pp:40-130.
29. Steel, R. G. and Torries, J. H. (1980). Principles and Procedures of Statistics. Abiometrical approach, 2nd edition. McGraw-Hill Book Co. New York, USA
30. Khan, M.R.; Khan, G.N and Ahmed, D. (2011). Evaluation of antioxidant and fertility effects of Digeramuricata in male rats. Afri. J. Pharm. Pharma., 5(6):688-699.
31. Castilla-Cortázar, I.; García, M. and Quiroga, J. (2000). Insulin-like growth factor-I reverts testicular atrophy in rats with advanced cirrhosis. Hepatology .,31: 592-600
32. Jorsaraei, A.G.S.; Firoozjaee, A.; Pasha, Y.Y.; Marzony, T.E. and Sarabi, E. M. (2010). Histopathological Effects of single dose treatment of diazinon on testes structure in rat. Yakhteh Med. J., 12 (1):39-42.
33. Cunningham, J. G. and Klein, B.G. (2007). Textbook of Veterinary Physiology. 4rd edition. Elsevier Saunders Company, USA, Pp:421-422
34. Ismail, H. and Al-nahari, H. (2009). Therapeutic and protctive role of Panax ginseng on pituitary and testicular axis in

male rats treated with carbon tetrachloride. Ale. J. Agric. Res., 54(1):1-12

35. Kim, N.D. and Mehta, R.; et al. (2002). Chemopreventive and adjuvant therapeutic potential of pomegranate (Punicagranatum) for human breast cancer. Breast Cancer Res. Treat., 71(3):203–217.
36. Adams, L.S.; Zhang, Y.; Seeram, N.P.; Heber, D. and Chen, S. (2010). Pomegranate ellagitannin - derived compounds exhibit antiproliferative and antiaromatase activity in breast cancer cells in vitro. Cancer Prev. Res., 3(1):108-113.
37. Osman _Eribas¸ A.; T_rk2, G.; Sçnmez, M.; Sakin, F. and Ates¸ A. (2010). Toxic effect of cyclophosphamide on sperm morphology, testicular histology and blood oxidant-antioxidant balance, and protective roles of lycopene and ellagic acid. basic. Clin. Pharm. Toxico.; 107: 730 -735.
38. Simanainen, U.; McNamara, K.; Davey, R.A.; Zajac, J.D. and Handelsman ,D.J.(2008). Severe subfertility in mice with androgenreceptor inactivation in sex accessory organs but not in testis. Endocrin., 149:3330–3338.
39. Zitzmann, M. (2008). Effects of testosterone replacement and its pharmacogenetics on physical performance and metabolism. Asian J .Androl., 10(3): 364-372.
40. Choi, S.M. and Lee, B.M. (2004). An alternative mode of action of endocrine-disrupting chemicals and chemoprevention. J. Toxicol .Environ Health B. Crit. Rev., 7(6): 451-463.
41. Chen, C.H.; Liu, T.Z.; Wong, C.H.; Lu, F.J. and Chen, S.C.(2007). The efficacy of protective effects of tannic acid, gallic acid, ellagic acid, and propyl gallate against hydrogen peroxide-induced oxidative stress and DNA damages in IMR-90 cells. Mol. Nutr. Food Res., 51(8):962-968.
42. Turk, G.; Sonmez, M.; Aydin, M.; Yuce, A.; Gur, S.; Yuksel, M.; Aksu, E. H. and Aksoy, H. (2007). Effects of pomegranate juice consumption on sperm quality, spermatogenic cell density, antioxidant activity and testosterone level in male rats. Clin. Nut., 27:289-296
43. Shyu, M.H.; Kao, T.C. and Yen, G.C. (2008). Prevents rat liver fibrosis induced by $CCl_4$ via inhibition of hepatic stellate cells activation. Food Chem. Toxicol., 46:3707-3713.
44. Dominic Amalraj, A.; Parkavi, C.; Murugaiah, K. and Dhanaraj, T.S. (2012). Hypolipidemic Activity of Cyperousrotundus on $CCl_4$ Induced Dyslipidemia in Rats. Asian J. Pharm. Tech., 2(2):51-53.
45. Buring, A.H. (1992). Purple grape juice improves endothelial functions reduces the susceptibility of LDL cholesterol to oxidation in patients with coronary artery diseases. Circulation. 45:1050-1055.
46. Esmaillzadeh, A.; Tahbaz, F. and Gaieni, I. (2006).Cholesterol-lowering effect of concentrated pomegranate juice consumption in type II diabetic patients with hyperlipidemia. Int. J. Vitam. Nutr. Res., 76:147-151.
47. Ibrahium, M.I. (2010). Efficiency of Pomegranate Peel Extract as Antimicrobial, Antioxidant and Protective Agents. J. Agricul. Sci., 6 (4): 338-344.
48. Tzulker, R.; Glazer, I.; Bar-Ilan, I.; Holland, D.; Aviram, M. and Amir, R. (2007). Antioxidant activity, polyphenol content and related compounds in different fruit juices and homogenates prepared from 29 different pomegranate accessions. J. Agric. Food Chem., 55: 9559-9570.
49. McLachlan, R.I.; O'Donnell, L.; Meachem, S.J.; Stanton, P.G.; de Kretser D.M.; Pratis, K. and Robertson, D.M.(2002). Identification of specific sites of hormonal regulation in spermatogenesis in rats, monkeys, and man. Rece. Prog. Horm. Res., 57: 149-179
50. Haywood, M.; Spaliviero, J.; Jimemez, M.; King, N.J.; Handelsman, D.J. and Allan, C.M. (2003). Sertoli and germ cell development in hypogonadal (hpg) mice expressing transgenic follicle-stimulating hormone alone or in combination with testosterone. Endocrin., 144: 509–517.
51. Mineka, Y.; Yuko, W. and Jun, Y. (2005). Inhibitory effect of an ellagic acid rich pomegranate extract on tyrosinase activity. Biosci. Biotechnol. Biochem., 69(12):2368-2373.

# الدور الوقائي لمستخلص قشور الرمان على خصى ذكور ألارانب البالغه المعاملة برابع كلوريد الكاربون

**وسن محمد حسين و جواد كاظم عراك**
فرع الفسلجة والادوية- كلية الطب البيطري- جامعة بغداد - العراق

## الخلاصة

تهدف الدراسة الحالية الى تحضيرمستخلص اثيلي من لب الرمان وتأثيره على نسبة وزن الخصى الى وزن الجسم , مستوى الكولستيرول في الدم , تركيز هرمون الشحمون الخصوري والتأثيرات النسجية المرضية على الخصى في ذكور الارانب البالغة المعاملة برابع كلوريد الكاربون.استعملت اربعه وعشرون من ذكور الارانب البالغه، قسمت بصوره عشوائية الى اربع مجاميع متساوية وعولجت لمدة 56 يوم كما يلي : المجموعة الاولى أعطيت 1 مل ماء مقطر يوميا و 0.5 مل/كغم وزن الجسم زيت الزيتون حقنا في البريتون مرتين في الاسبوع واعتبرت مجموعه سيطرة . المجموعة الثانيه اعطيت مستخلص لب الرمان 100ملغم /كغم وزن الجسم عن طريق الفم يوميا و 0.5 مل/كغم وزن الجسم زيت الزيتون حقنا في البريتون مرتين في الاسبوع (المجموعة المعالجة الاولى) . المجموعة الثالثه اعطيت 500 ملغم /كغم وزن الجسم من رابع كلوريد الكاربون مخلوطه مع كميه متساويه من زيت الزيتون حقنا في البريتون مرتين في الاسبوع (المجموعه المعالجة الثانية). المجموعة الرابعة اعطيت مستخلص لب الرمان 100 ملغم / كغم من وزن الجسم يوميا عن طريق الفم اضافه الى 500 ملغم /كغم وزن الجسم من رابع كلوريد الكاربون مخلوطه مع كميه متساويه من زيت الزيتون حقنا في البريتون مرتين في الاسبوع (المجموعة المعالجة الثالثة). تم سحب الدم في الايام ( 0و14 و28 و42 و56) لحساب تركيز هرمون الشحمون الخصوي ومستوى الكولسترول في الدم. في نهاية التجربه وزنت الارانب وتم عزل الخصى ووزنت لغرض معرفة نسبه وزن الخصى الى وزن الجسم واجراء الفحص النسجي المرضي للخصى. اظهرت النتائج ان اعطاء مستخلص لب الرمان اظهر زياده معنويه في نسبة وزن الخصى الى وزن الجسم بينما اظهرت نقصان معنوي في مستوى الكولسترول وارتفاعا معنويا في تركيز هرمون الشحمون الخصوي, وأظهر الفحص النسجي المرضي إن مستخلص لب الرمان وقى الخصى من الافات التي سببها التعرض لرابع كلوريد الكاربون. نستنتج من هذه الدراسة ان مستخلص لب الرمان له القدره على حماية نسيج الخصى المعرض لرابع كلوريد الكاربون وقد يعود السبب الى الفعل المضاد للاكسدة للب الرمان وبالتالي القضاء على الاثار الظارة لسمية رباعي كلوريد الكاربون.

**الكلمات المفتاحية: مستخلص قشور الرمان , خصى ذكور الارانب , رباعي كلوريد الكاربون.**