The effect of pomegranate juice and peel on testosterone level and antioxidants activity in male rats

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Abstract

Pomegranate (*Punica granatum* L.) has been proved as having value in managing male sexual disorders. This purpose of this study is studiding the effect of pomegranate juice and peel on testosterone level and antioxidants activity in male rats.Minerals content, vitamins , fractions of polyphenols and were determined. The results showed that the pomegranate peel and juice had contained the highest amounts of Minerals content, vitamins and natural antioxidant compounds. Thirty-six adult male rats were divided into six groups, with six rats each group. Experimental rats were fed on basal diet for 15 days and randomly divided into six groups six rats for each. The 1st main group was fed on basal diet for another 6 weeks and considered as control negative rats .The other five groups administered 7 ml /kg cisplatin / kg. "positive control" the third group fed on basal diet and Served as (0.25 ml pomegranate juice/ rat) by oral tube.The fourth group fed on basal diet and received oral administration of 0.75 mL pomegranate juice /rat. The fifth group fed on basal diet and received 10 gm powder pomegranate peel /rat. the final group fed on basal diet and received oral administration of 20 gm powder pomegranate peel. At the end of biological experiential (five weeks) the gain body weight was recorded every three days for five week. Moreover, the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera after that, the sera were kept in a deep - freezer at -20°C until their analysis. Tests were dissected out and quickly frozen in liquid N2 for histological examination .The tissue specimens from tests were collected from treated and control rats by the end of the experiment. The results showed that the pomegranate juice contained P, Mn, Fe, and Cu at the level of 7.82, 5.12, 3.37, and 0.71 mg/100g dry matter respectively. The results observed that the pomegranate peel powder contained B1 (thiamine), B2 (riboflavin), C (L-ascorbic acid), E (α-Tochoferol) and A (Retinol) at the levels of 0.131, 0.11, 13.4, 3.25 and 0.15 mg/100g dry matter, versus 1.42, 0.97, 15.13, 1.79 and 0.09 mg/100g dry matter for pomegranate juice, respectively. The results indicated that the antioxidant enzymes GSH, G-PX, and CAT were the lowest in the cisplatin group (2) (14.08 mmol/l, 9.58 mmol/l, and 9.09 u/mg, respectively) which injection with cisplatin and fed on basal diet than other groups. Meanwhile, the control healthy group the highest in the antioxidant enzymes was 96.88 mmol/l, 79.11

mmol/l, and 12.20 u/mg, respectively, and fed on basal diet. The different cisplatin groups treated with pomegranate juice and peel powder had the best results thus show the rat's group (4) which taken orally one milliliter daily contained 0.50 mL pomegranate juice plus 0.50 mL distilled water and fed on a basal diet gave 43.63 mmol/l, 37.31 mmol/l and 9.72 u/mg, respectively. Moreover, the results from the group (6) which fed on basal diet substituted with 20g pomegranate peel give 58.59 mmol/l, 67.16 mmol/l, and 10.58 u/mg, respectively the best results when the rats fed on a high amounts and orally from pomegranate peel and juice and give the best results .In addition ,the results it could be noticed that all parameters especially fertility improvements and give the best results. Moreover, the histological examination of testis was confirmed .It could be noticed that the pomegranate juice and the peel were improvement all parameters especially fertility and sexual tract. Therefore, it could be recommended that pomegranate juice and the peel is a good source for health sexual.

Keywords: juice and peel pomegranate, fertility, cisplatin, spermatogoni, main primary spermatocyte, many spermatozoa.

Introduction

Pomegranate (Punica granatum) has been used in the folk medicine of many countries especially in the Middle East ***(Gurib-Fakim A,2006*)**. Edible parts of pomegranate fruit show 52% of total fruit weight, comprising 78% juice and 22% seeds. Pomegranate is rich in antioxidant of polyphenolic which includes tannins and anthocynins and flavonoids **(de Nigris et al., 2007; Ricci et al., 2006).**The content of soluble polyphenols in pomegranate juice varies within the limits of 0.2% to 0.1% including mainly tannins, ellagic tannins, anthocyanins, catechins, gallic and ellagic acids **(Gil et al., 2000).** There are many evidences that flavonoids interact with various biological system **(Lairon and Amiot, 1999)**. It is widely seen that pomegranate exhibits antivirus, antioxidant, antidiabetic, antidiarrheal, anti- cancer and antiproliferative activities ***(Faria et al., 2006;Abdel Moneim, 2012).***

it was noticed that peel pomegranate has significant effect on some oxidants/antioxidants enzymes of liver and kidney when compared to the control. (***Abdel Moneim, 2011).***

Reactive oxygen species (ROS) are very reactive oxidizing agents belonging to the class of free radicals. The production of ROS in various organs including the testis is a normal physiological event; however, the alterations in their synthesis stimulate the oxidation and DNA damage of cells ***(Sikka SC, 1996).***The plasma membrane of sperms contains a high amount of unsaturated fatty acids. Thus, it is particularly susceptible to peroxidative damage. The lipid peroxidation destroys the structure of the lipid matrix in the membranes of spermatozoa, and it is associated with loss of motility and the defects of membrane integrity ***(Henkel R, 2005).***

Antioxidants, in general, are compounds which dispose, scavenge, and suppress the formation of ROS and lipid peroxidation. Among the well-known biological antioxidants, glutathione (GSH), glutathione peroxidase (GSH-Px), catalase (CAT), superoxide-dismutase (SOD) have a significant effect as a suppressor or scavenger of free radicals. Hence, the application of ROS scavengers is likely to improve sperm function ***(Vernet P, Aitken RJ, Drevet JR, 2004).***

Chemotherapy for testicular cancer dependent on cisplatin (CP) or cisdia mine can also result in impaired spermatogenesis ***(Cherry et al., 2004),*** c hromosome sperm defects ***(Martin et al., 1999),*** and temporary or permanent azoospe rmia **(Howell and Shalet, 2001).**The use 0f cisplatin CP for clinical purposes is limited by its side effects, and toxic t o the male reproductive system has been reported. It has been observed that male rats treated with CP have decreased reproductive organ weig hts and impaired fertility along with changes in nextgeneration growth an d development ***(Cherry et al., 2004).*** In addition, humanbased studies have shown encouraging results a nd suggested the ability of pomegranate as a protective agent for many disea ses ***(Kandylis and Kokkinomagoulos, (2020).*** Pomegranate fruit can be eaten pure or used to produce pomegranate juice, and any diet is a good addition to that. Both the pome granate seeds and the surrounding pulp are nutritious and edible ***(Avdat ek et al. , 2018).***

Pomegranate fruit, juice, and peel possess a marked antioxidant capacity ***(Kaur et al., 2006)*** wita high content in polyphenols, in particular, ellagita nnins is condensed tannins and anthocyanins ***(Seeram et al., 2005)***. Any of these a ntioxidntmolecules were shown to be healthy and bioavailable ***(MertensT alcott et al., 2006)***. Granate juice intake significantly increases sperm pro duction, sperm cell density, antioxidant .The current study aims to evaluate the beneficial effect of juice and peel pomegranate on testosterone level and antioxidants activity on male rats and histological testis that may makes it one of the most important foods for the future.

Materials and Methods:

Materials:

fresh pomegranate (Punica granatum L.) fruit were purchased from local markets.in Egypt.Cisplatin drug was purchased in vial containing 50 mg o f powder dissolved in 50 ml solution manufactured by Merck / France, Bi con Diagnosemittel GmbH und Co got kits of different parameters.

Methods:

Preparation of pomegranate peels powder and juice:

Pomegranate fruits were washed with distilled water then peeled, and Car efully
separated their edible portions (seeds). The peels were air.dried for 48 h i n a ventilated oven at 40 ° C and ground to a fine powder and passed thro ugh a mesh sieve
and stored immediately before analysis at 20° C.Pomegranate juice was prepared
as follows: the edible portions (seeds) of the pomegranate fruit containing the intact
extract sacs were manually removed from the pericarps and sacks and split in a
5:10 sec electric blender by very light agitation. The resultant extract was then centrifuged for 10 min at 1,400 g. The supernatants from the pomegr anate juice centrifugation step were recovered, filtered, aliquot, and store d at 20º.

Biological experimental :

Animals and experimental design:

Wister albino weaning rats (36 rats) with a weight range from 120-130 g. were obtained
from the National Organization for Drug and Control Research, Giza,- Egypt. Rats were housed in individual cages with screen bottoms and fed adlibitum on a basal diet for one-week for acclimatization, which containing casein (20 %), corn oil (8%), corn starch (31%), sucrose (32%), cellulose (4%), salt mixture (4%) and vitamin mixture (1%) according to the method **Pell *et al*. (1992).**

Experimental rats were fed on basal diet for 15 days and randomly divided into six groups six rats for each. The 1st main group was fed on basal diet for another 6 weeks and considered as control negative rats.The other five groups administered 7 ml /kg cisplatin / kg. “positive control” the third group fed on basal diet and Served as (0.25 ml pomegranate juice/ rat) by oral tube (administration via epigastria tube) . The fourth group fed on basal diet and received oral administration of 0.75 mL pomegranate juice /rat. The fifth group fed on basal diet and received oral administration of 10 gm powder pomegranate peel /rat. the final group fed on basal diet and received oral administration of 20 gm powder pomegranate peel.

At the end of biological experiential (five weeks) the gain body weight was recorded every three days for five week. Moreover, the blood samples were taken with drawn from the orbital plexus and centrifuged at 3000 rpm to obtain the sera after that, the sera were kept in a deep - freezer at -20°C until their analysis. Tests were dissected out and quickly frozen in liquid N2 for histological examination .The tissue specimens

from tests were collected from treated and control rats by the end of the experiment.

Determination of Kidney functions

Kidney functions as creatinine and urea were estimated according to the method described by ***Schirmeister (1964) and Patton and Crouch (1977)***

Determination of vitamin levels in plasma

Vitamin A and E antioxidant levels from plasma, 100 mL of plasma was deproteinized with 100 mL of ethanol, and 600 mL of chloroform was ext racted.

The extract was shaken for 5 minutes before centrifuging for 8 minutes at 12 000 rpm. The organic layer under nitrogen was removed and evaporat ed ***(Zhao et al., 2004).***

Plasma testosterone

The level of plasma testosterone was determined using the ELISA metho d using a DRG Elisa testosterone kit (ELISA EIA1559, 96 Well kit, DRG Instruments,

GmbH, Marburg, Germany) as instructed by the kit manufacturer ***(Türk et al . , 2008***).

Polyphenolic fractions of pomegranate peel powder and juice:

Polyphenolic compounds in of pomegranate peels powder and pomegranate juice were fractionated using HPLC apparatus

Determination of lipid peroxidation and antioxidant enzyme

Lipid peroxidation determination and antioxidant enzyme determination

The lipid peroxidation was colorimetrically evaluated by ***Yoshioka et al . (1979)*** as malondialdehyde (MDA).

Determination of minerals and vitamins content in pomegranate peel and juice:

Macroelement (calcium) and Microelements (iron, manganese, selenium and copper) of pomegranate peel and juice were determined according to the method of the **AOAC (2010),** using Atomic Absorption Spectrophotometer (Perkin Elmer, Model 3300, Germany). Phosphorus was determined by spectrophotometer according to the **AOAC (2010),** while sodium and potassium contents were determined by Flame Photometer (CORNING 400, serial No. 4889.UK).

Vitamins B1 (Thiamin), B2 (riboflavin) and *L*-Ascorbic acid vitamin C of pomegranate peel and juice were determined according to the method described in the **AOAC (2010)** using High Performance Liquid Chromatography (HPLC) Beckman model equipped by double piston pump 126 with Fluorescence detector LC 240 (Perkin Elmer). Whereas, Vitamin E (α-tocopherol) and Vitamin A (Retinol) was

measured by using high pressure liquid chromatography (HPLC) method described by **Leth and Sondergaro (1983)** and **Leth and Jacobsen (1993).**

Determination of polyphenolic fractions from pomegranate peel and juice:

HPLC technique was used for separation and estimation of polyphenolic compounds in pomegranate peel and juice were determined according to the method described by **Madrigal-Carballob *et al.* (2009).** HPLC instrument (Hewlett Packard series 1100 HP) Column hypersil BDS 5 µm C 18 and Detector UV 254 nm.

Histological examination

For histological studies, the testis was fixed overnight in Bouin's fluid, dehydrated nethanol,and embedded inparaffin. Sections of tissue (6 µm thick) were cut to a microtome, moun ted on a glass slide.

Statistical analysis

The obtained data were exposed to the analysis of variance. Duncan's multiple range tests at ($P \leq 0.05$) level was used to compare between means. The analysis was carried out using the ANOVA procedure of Statistical Analysis System **(SAS, 2004).**

RESULTS AND DISCUSSION

Minerals and vitamins content in pomegranate peel and juice:

The pomegranate peel powder and juice with regards to their minerals content were estimated and the obtained results are recorded as in Table (1). The results showed that the pomegranate peel contained the predominant minerals in it were found to be Ca, K, Na, and P at the level of 234.1, 152.0 74.8, and 20.4 mg/100g dry matter; respectively. In addition, the pomegranate peel powder contained Fe, Zn, and Cu at the level of 2.12, 1.01, and 0.90 mg/100g dry matter; respectively.

The results in the same table showed that the major macroelements in the pomegranate juice were Na, Ca, and K which were found at the level of 501.3, 312.7and 139.12 mg/100g dry matter; respectively. Also, the pomegranate juice contained P, Mn, Fe, and Cu at the level of 7.82, 5.12, 3.37, and 0.71 mg/100g dry matter respectively. In general, it could be concluded that pomegranate peel and juice were considered a good source of macro and microelements.

Iron is an important trace element in the human body. It plays crucial roles in haemopoiesis, control of infection and cell mediated immunity **Beard, (2001).**The deficiency of iron has been described as the most prevalent nutritional deficiency and iron deficiency anemia is estimated to affect more than one billion people worldwide **Trowbridge and Martorell (2002).** Zinc is an essential micronutrient for human growth and immune functions **Black (2003).** Manganese (Mn) plays an

important role in a number of physiological processes as a constituent of some enzymes and an activator of other enzymes **Nielsen (1999).**

Essential dietary minerals assist in the regulation of fluid balance, muscle contractions, and nerve impulses. Minerals are absorbed through the intestine and the body usually regulates mineral stores to keep them in balance. **(Carolyn 2002).**

The vitamins including B1 (thiamine), B2 (riboflavin), C (L-ascorbic acid), E (α-Tochoferol), and A (Retinol) content of pomegranate peels powder and pomegranate juice were evaluated and the results were recorded in Table (1). The results observed that the pomegranate peel powder contained B1 (thiamine), B2 (riboflavin), C (L-ascorbic acid), E (α-Tochoferol) and A (Retinol) at the levels of 0.131, 0.11, 13.4, 3.25 and 0.15 mg/100g dry matter, versus 1.42, 0.97, 15.13, 1.79 and 0.09 mg/100g dry matter for pomegranate juice, respectively. The determined vitamins naturally occurred in pomegranate peels and juice are considered one of the most important with having the anti-oxidant, anti-microbial and chemo preventive cancer properties and good standpoint in human nutrition ***(Huxley and Neil, 2003).***

Table (1): Minerals and vitamins content of pomegranate peel and juice

Minerals and vitamins content (mg/100g)	Pomegranate peel	Pomegranate juice
Ca	**234.1±20.24**	**3212.7±30.54**
K	**152.0±15.28**	**139.12±12.68**
Na	**74.8±7.35**	**451.3±40.35**
P	**20.4±±2.79**	**7.82±0.82**
Fe	**2.12±0.91**	**3.37±0.26**
Mn	**1.01±0.12**	**5.12±0.27**
Cu	**0.90±0.07**	**0.71±0.04**
Thiamin (B1)	**0.131±0.02**	**1.42±0.09**
Riboflavin (B2)	**0.11±0.02**	**0.97±0.08**
***L*-Ascorbic acid (C)**	**13.4±1.24**	**15.13±1.57**
E (α-Tochoferol)	**3.25±0.71**	**1.79±0.02**
A (Retinol)	**0.15±0.01**	**0.09±0.001**

Each value represents the mean of three replicates ±SD

Polyphenolic fractions of pomegranate peel powder and juice:

Polyphenolic compounds in of pomegranate peels powder and pomegranate juice were fractionated using HPLC apparatus and the results are reported in Table (2). From the results it could noticed that the pomegranate peels and pomegranate juice had contained the gallic, ellagic, ρ-cumaric, ferulic, catechin, delphinidin, O – Cumaaric, caffeic, sinapic, luteolin, quercetin and kaempferol. Pomegranate juice was the highest values of the polyphenolic compounds than pomegranate peels. The results observed that the pomegranate peels powder and pomegranate

juice were increased in catechin, gallic acid, kaempferol, quercetin, ellagic acid, delphinidin, cyaniding and ferulic acid were 205,115,87, 65, 43, 59, 51 and 40 mg/100g, respectively pomegranate juice. Whereas, pomegranate peel powder had contained 231, 146, 125, 102, 91, 88, 82 and 72 mg/100g, respectively. Whilst, luteolin, caffeic acid, sinapic acid, O-cumaaric and P-cumaaric were the lowest amounted compounds in pomegranate peels powder and juice.

Several studies have shown that pomegranate exhibits high anti-oxidant activity. Pomegranate extracts have been shown to exhibit a 2–3-fold greater anti-oxidant effect than green tea **(Gil *et al.*, 2000)** and a 6–8-fold greater effect than grape, grapefruit and orange juice **(Tzulker *et al.*, 2007).**

Table (2): Percent of polyphenols content (mg/100g) in pomegranate peel and juice:

Polyphenols	Retention time	Pomegranate Peel	Pomegranate Juice
Kaempferol	**36.29**	**125±10.35**	**87±9.14**
Quercetin	**32.21**	**102±10.04**	**65±7.32**
Luteolin	**31.41**	**67±6.28**	**58±6.17**
Sinapic acid	**29.37**	**64±6.39**	**42±5.36**
Cyanidin	**28.03**	**82±9.51**	**51±4.76**
Caffeic acid	**25.72**	**66±6.33**	**36±2.59**
O – Cumaaric	**25.24**	**63±6.27**	**46±4.35**
Delphinidin	**24.01**	**88±8.94**	**59±6.18**
Catechin	**22.97**	**231±20.15**	**205±21.36**
Ferulic acid	**22.47**	**72±8.13**	**40±5.26**
P – Cumaaric	**21.03**	**53±4.28**	**57±6.19**
Ellagic acid	**16.38**	**91±10.24**	**43±4.12**
Gallic acid	**7.31**	**146±15.26**	**115±11.38`**

Each value represents the mean of three replicates ±SD

Effects of pomegranate peel powder juice on body weight initial, final, and gain in rats treated with cisplatin.

After five weeks Table (3) indicated that the final body weight of cisplatin-treated rats (control negative) appeared a significant reduction from 210.7 to 180.4g compared with the control of positive was 200.6 to 240.8g. The reduction in the body weight in CP-treated rats might be due to the direct toxic effect of CP on renal tubules, with subsequent polyuria and dehydration (**Yao *et al.*, 2007 and Azu *et al.*, 2010),** or due to gastrointestinal toxicity with a subsequent decrease in appetite, ingestion, and assimilation of food **(Arhoghro *et al.*, 2012).**

Moreover, the pomegranate juice at the highest doses rat's group (4) which taken orally one milliliter daily contained 0.50 mL pomegranate juice plus 0.50 mL distilled water and fed on a basal diet

was an improvement the final body weight and recorded 200.5 to 190.2g. Whilst, the results from the group (6) which fed on basal diet substituted with 20g pomegranate peel give the best results and recorded 207.7 to 200.1g in the final body weight. The pomegranate peel powder and juice have a powerful antioxidant activity and improved the weight loss effects.

These results confirmed by **Les *et al.* (2017 and 2018)** who found that the pomegranate juice and peel components may be related to the diverse phenolic and flavonoids compounds that have presented the ability to inhibit lipase, triglyceride accumulation, and adipogenesis-related genes, as well as to decrease lipogenesis and lipolysis in mouse and human adipose cells, therefore, it could to be used as a functional food for the prevention of diseases that are associated with weight loss, diabetes, and dyslipidemias**.**

Table (3): Body weight (gm), gain%, food intake (gm) and food efficiency ratio of rats fed on pomegranate peel powder juice.

Treatments	Initial body weight g	Final body weight g	Gain body weight g	Daily gain body weight
Control negative	**200.6±7.26**	**240.8±12.39**	**+ 40.2±0.91**	**1.15±0.05**
Control positive	**210.7±9.13**	**180.4±6.81**	**- 30.3±0.63**	**- 0.87±0.02**
Group (3) 0.25mL juice	**190.6±8.62**	**170.2±5.64**	**- 20.4±0.26**	**- 0.58±0.02**
Group (4) 0.75mL juice	**200.5±11.86**	**190.2±9.28**	**- 10.3±0.65**	**- 0.29±0.01**
Group (5) 10.0g peel	**205.7±10/57**	**190.2±9.37**	**- 15.2±0.43**	**- 0.43±0.01**
Group (6) 20.0g peel	**207.7±6.89**	**200.1±10.28**	**- 7.6±0.27**	**- 0. 22±0.01**

Values are mean and SD (n = 6);

Pomegranate peels powder and juice protect antioxidant vitamins in plasma and plasma testosterone from cisplatin

Table (4) showed that the effect of cisplatin on antioxidant vitamins in plasma and plasma testosterone on different rats groups and these groups treated with pomegranate peels powder and juice at different levels. From the results, it could be noticed that when these cisplatin groups treatment with pomegranate peels powder and juice the plasma vitamins are protected against detrimental effects of CP, these may be due to pomegranate peels powder and juice which had contained a strong natural antioxidant.

The results from plasma testosterone in the same table showed that the cisplatin effect in the group rats control positive was the lowest (0.47ng/ml) than the control negative healthy group rats was the highest (2.29 ng/ml). Moreover, the pomegranate juice at the highest doses rat's group (4) which taken orally one milliliter daily contained 0.50 mL pomegranate juice plus 0.50 mL distilled water and fed on a basal diet was an improvement the plasma testosterone and recorded 1.33ng/ml. While, the results from the group (6) which fed on basal diet substituted

with 20g pomegranate peel give the best results and recorded 1.94ng/ml in plasma testosterone. These results confirmed with **Abarikwu *et al.*, (2012)** who found that the CP administration significant decrease in intra-testicular testosterone concentration 3β- Hydroxysteroid dehydrogenas 3β-HSD) and 17β- Hydroxysteroid dehydrogenas 17β-HSD) are the main enzymes in testicular androgenesis and play a key regulatory role in testicular steroidogenic events. The low level of these enzymes by cisplatin treatment might be the cause of decreased testosterone concentration**.**

The effects of CP on sperm have been linked to the oxidative stress-inducing potentials of the compound. CP induced ROS generation in the testis and sperm and caused cell death in the seminiferous epithelium (**Aksu *et al.*, 2017).**

Table (4): Effect of cisplatin on antioxidant vitamins in plasma and plasma testosterone

Treatments	Vit A	Vit E	Vit C	Plasma testosterone (m mol/mg)
Control negative	**4.99 ±0.73^{a}**	**4.09 ±0.76^{a}**	**7.10 ±0.84^{a}**	**2.29 ±0.28^{a}**
Control positive	**0.99 ±0.05^{c}**	**0.62 ±0.57^{c}**	**2.01 ±0.43^{d}**	**0.47 ±0.39^{c}**
Group (3) 0.25 mL juice	**2.02 ±0.36 ^{b}**	**1.08 ±0.11^{ab}**	**2.76 ±0.31^{b}**	**0.91 ±0.81^{b}**
Group (4) 0.50 mL juice	**2.71 ±0.31^{b}**	**2.77 ±0.21 ^{ab}**	**3.87 ±0.41 ^{cb}**	**1.33 ±0.74^{ab}**
Group (5) 10.0g peel	**2.02 ±0.34 ^{b}**	**1.88 ±0.28^{b}**	**3.00 ±0.39^{cb}**	**1.00 ±0.92 ^{ab}**
Group (6) 20.0g peel	**4.03 ±0.69 ^{a}**	**4.51 ±0.79 ^{a}**	**5.91 ±0.49^{b}**	**1.94 ±0.76 ^{a}**

Pomegranate peels powder and juice protect kidney functions from cisplatin

Cisplatin (CP), as an antitumor drug, is widely used for chemotherapy (**Florea and Büsselberg, 2011).** CP-induced nephrotoxicity is related to its accumulation in the proximal tubule cells, (**Townsend *et al.*, 2003)** and synthetic or herbal agents have been investigated as supplementations against CP-induced nephrotoxicity (**Atessahín *et al.*, 2007).**

The effect of the treatment of rats with pomegranate peel powder and juice to improve the level of serum creatinine and urea in different group rats injected with cisplatin and the results are shown in Table (5). From the results, it could be indicated that the creatinine and urea were the highest in the cisplatin group (3.25 and 101.76 mg/dl), and also, the control healthy group rats give the lowest results a normal kidney function (0.42 and 42.0 mg/dl), respectively. The different cisplatin groups treated with pomegranate juice and peel powder had the best

results thus show the rat's group (4) which taken orally one milliliter daily contained 0.50 mL pomegranate juice plus 0.50 mL distilled water and fed on a basal diet, the creatinine, and urea give 1.03 and 66. 96 mg/dl. Moreover, the results from the group (6) which fed on basal diet substituted with 20g pomegranate peel gave 0.81 and 52.53 mg/dl, respectively. These results confirmed with **Bakır *et al.* (2015)** demonstrate that the anti-oxidant pomegranate juice might have a protective effect against cisplatin-induced toxicity in rat kidney, but not in liver. Pomegranate juice could be beneficial as a dietary supplement in patients receiving chemotherapy medications.

Some previous studies showed that some antioxidant agents have a protective effect against CP-induced nephrotoxicity in males but not in females (**Eshraghi-Jazi *et al.*, 2013)** while nephrotoxicity induced by CP may be gender-related, (**Nematbakhsh *et al.*, 2013)** and presence of estrogen aggravates nephrotoxicity induced by CP in ovariectomized female rats (**Pezeshki *et al.*, 2013).**

Table (5): Effect of cisplatin on kidney functions

Treatments	Creatinine (mg/dl)	Urea (mg/dl)
Control negative	**0.42±0,02^{d}**	**42.00±4.12^{e}**
Control positive	**3.25± 0.74^{a}**	**101.76±10.35^{a}**
Group (3) 0.25 mL juice	**2.05±0,53^{b}**	**74.81±7.26^{b}**
Group (4)0.50 mL juice	**1.03±0,20^{c}**	**66.96±6.81^{c}**
Group (5) 10.0g peel	**1.03±0,21^{c}**	**69.01±7.19^{bc}**
Group (6) 20.0g peel	**0.81±0,04^{d}**	**52.53±5.46^{d}**

Values are mean and SD (n = 6); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Pomegranate peels powder and juice protect antioxidant enzymes from cisplatin

Lipid peroxidation as Malondialdehyde (MDA) and the antioxidant enzymes contained Catalase (CAT) Glutathione Peroxidase (G-PX) and Glutathione (GSH) were determined in plasma at the end of the experimental period (six weeks) after injection with cisplatin in male rats and treatment with pomegranate peels powder and juice at different levels and the results are reported in Table (6).

The results indicated that the antioxidant enzymes GSH, G-PX, and CAT were the lowest in the cisplatin group (2) (14.08 mmol/l, 9.58 mmol/l, and 9.09 u/mg, respectively) which injection with cisplatin and fed on basal diet than other groups. Meanwhile, the control healthy group the highest in the antioxidant enzymes was 96.88 mmol/l, 79.11 mmol/l, and 12.20 u/mg, respectively, and fed on basal diet. The different cisplatin groups treated with pomegranate juice and peel powder had the best results thus show the rat's group (4) which taken orally one milliliter daily contained 0.50 mL pomegranate juice plus 0.50 mL distilled water and fed on a basal diet gave 43.63 mmol/l, 37.31 mmol/l and 9.72 u/mg,

respectively. Moreover, the results from the group (6) which fed on basal diet substituted with 20g pomegranate peel give 58.59 mmol/l, 67.16 mmol/l, and 10.58 u/mg, respectively. These results administration of pomegranate juice and peel powder at different levels caused a significant increase in the activity of CAT enzymes when compared to the control group. The improvement of CAT enzyme activities could be explained by antioxidant properties of Pomegranate seeds extract due to the presence of bioactive polyphenolic compounds that play a role in scavenging free radicals and also prevent DNA damage **(Rom *et al.*, 2016).** In addition, **Rouh*i et al.* (2017)** demonstrated that pomegranate afforded up to 60 % protection against hepatic lipid peroxidation due to maintenance of the GSH and serum levels and activities of CAT, GPx, and glutathione reeducates (GR) enzymes.

Similarly significant decrease in the level of SOD activity was observed in CP treated group that are in accordance with previous studies in which CP treatment caused reduction in the levels of antioxidant enzymes SOD is important in cell defense mechanisms because SOD converts the superoxide ions to elemental oxygen and hydrogen peroxide (**Wei *et al.*, 2011).**

The results from Lipid peroxidation as Malondialdehyde (MDA) in the same table were parallel and confirmed the results from antioxidant enzymes. This was probably due to pomegranate peels powder and juice direct antioxidant effects or the enhanced biosynthesis of GSH and the other antioxidant enzymes **(Ghanbarzadeh *et al.*, 2014).** In addition, pomegranate peels powder and juice reduces the availability of lipids for peroxidation by transporting fatty acids into the mitochondria for β-oxidation and consequently mitigates the production and accumulation of lipid peroxidation products **(Dokmeci *et al.*, 2005 and Derin *et al.*, 2006).** Moreover, the pomegranate peels powder and juice reduced oxidative stress through attenuation of MDA production and improvement of the antioxidant status in testicular tissues via augmentation of SOD, CAT, GPx, and GSH levels. Our results were in harmony with earlier reports showing that pomegranate peels powder and juice attenuated lipid peroxidation and enhanced the antioxidant balance in rat testicular tissues **(Yuncu *et al.*, 2015).**

Table (6): Effect of cisplatin on antioxidant enzymes

Treatments	GSH (m mol/mg)(	G- Px (m mol/mg)	MDA)m mol/mg(	CAT) u/mg (
Control negative	96.88 ±8.25[a]	79.11 ±7.26[a]	12.20 ±1.53[a]	0.50 ±0.01[e]
Control positive	14.08 ±2.04[e]	9.58 ±1.22[e]	5.09 ±0.43[c]	9.09 ±1.32[a]
Group (3) 0.5mL	22.19 ±2.46[d]	20.04 ±2.38[d]	8.69 ±0.67[b]	4.68 ±0.53[b]
Group (4) 1.0ml	43.63 ±3.59[c]	37.31 ±3.86[c]	9.72 ±0.76[ab]	2.67 ±0.11[c]
Group (5) 5.0g p	26.64 ±3.12[d]	27.61 ±2.27[d]	9.48 ±1.08[ab]	3.49 0.21[bc]
Group (6) 10.0g	58.59 ±5.28[b]	67.16 ±6.51[b]	10.58 ±1.24[a]	1.00 ±0.08[d]

Values are mean and SD (n = 6); where: Mean values in the same with the letter are significantly different at 0.05 levels.

Histopathological results:

Testis: at 0.05 levels.

Histopathological testis:

Figure (1 and 2) control group (1): testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining up to complete spermatogenesis, and average interstitium with average leydig cells.

Figure (3 and 4) group (2) control cisplatin: testis showed thick tunica albuginea, widely-spaced small-sized distorted tubules with markedly thickened basement membrane, marked reduction of germinal lining and no spermatids and spermatozoa in some tubules , and average interstitium showing Leydig cells.

Figure (5 and 6) group (3): testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining with full spermatogenesis, scattered tubules with mildly thickened basement membrane, and mildly edematous interstitium with average Leydig cells.

Figure (7 and 8) group (4): testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining with full spermatogenesis, few scattered tubules with mildly thickened basement membrane, and average interstitium with average Leydig cells.

Figure (9 and 10) group (5): testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with thin detached germinal lining with full spermatogenesis, scattered tubules with mildly thickened basement membrane and reduction of spermatogenesis, and markedly edematous interstitium with dilated thrombosed interstitial blood vessels.

Figure (11 and 12) group (6): testis showed average tunica albuginea, average sub-capsular blood vessels, average sized tubules with average germinal lining with full spermatogenesis, few scattered distorted tubules with mildly thickened basement membrane, and average interstitium with average Leydig cells.

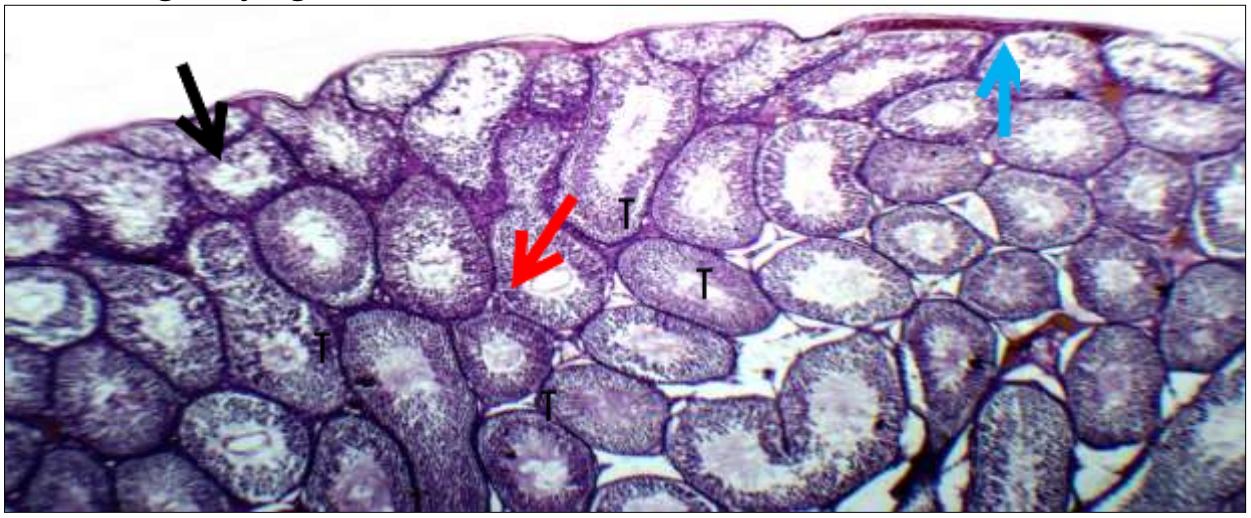

Fig 1: Control: slide G1: testis showing average tunica albuginea (black arrow), average sub-capsular blood vessels (blue arrow), average sized tubules (T), and average interstitium (red arrow) (H&E X100)

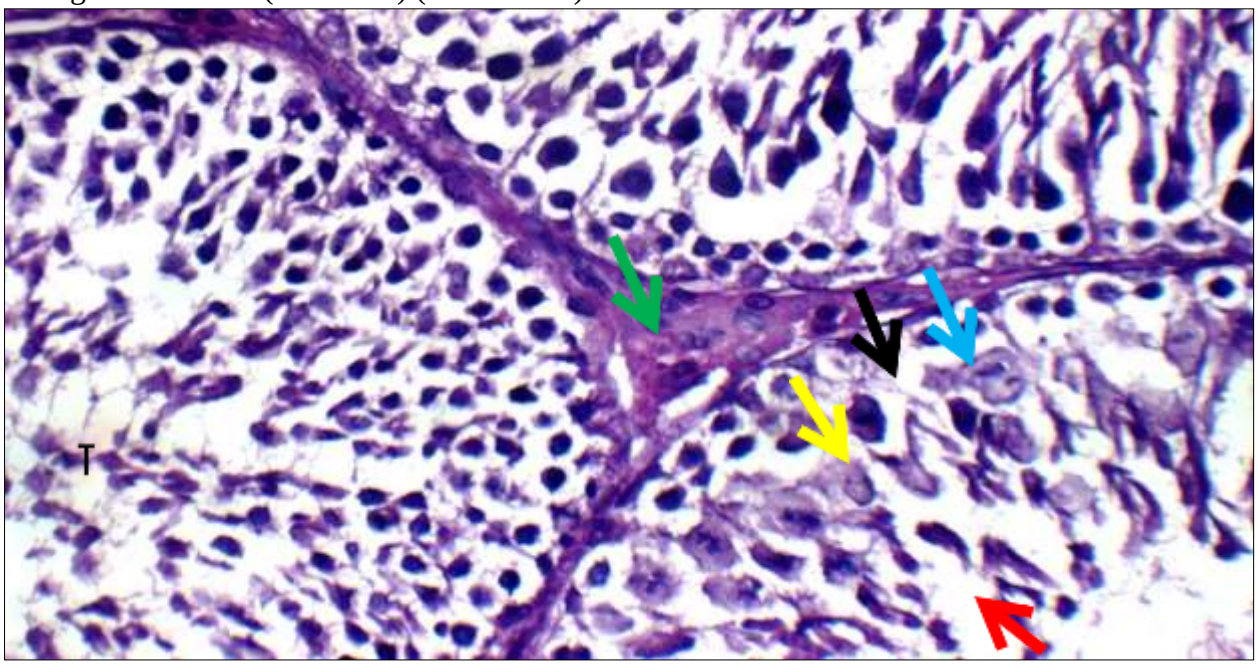

Fig 2: Control: slide G1: higher power view showing tubules with average basement membrane (black arrow), spermatogonia (blue arrow), primary spermatocyte (yellow arrow), many spermatozoa (red arrow) and average interstitium showing Leydig cells (green arrow) (H&E X 400)

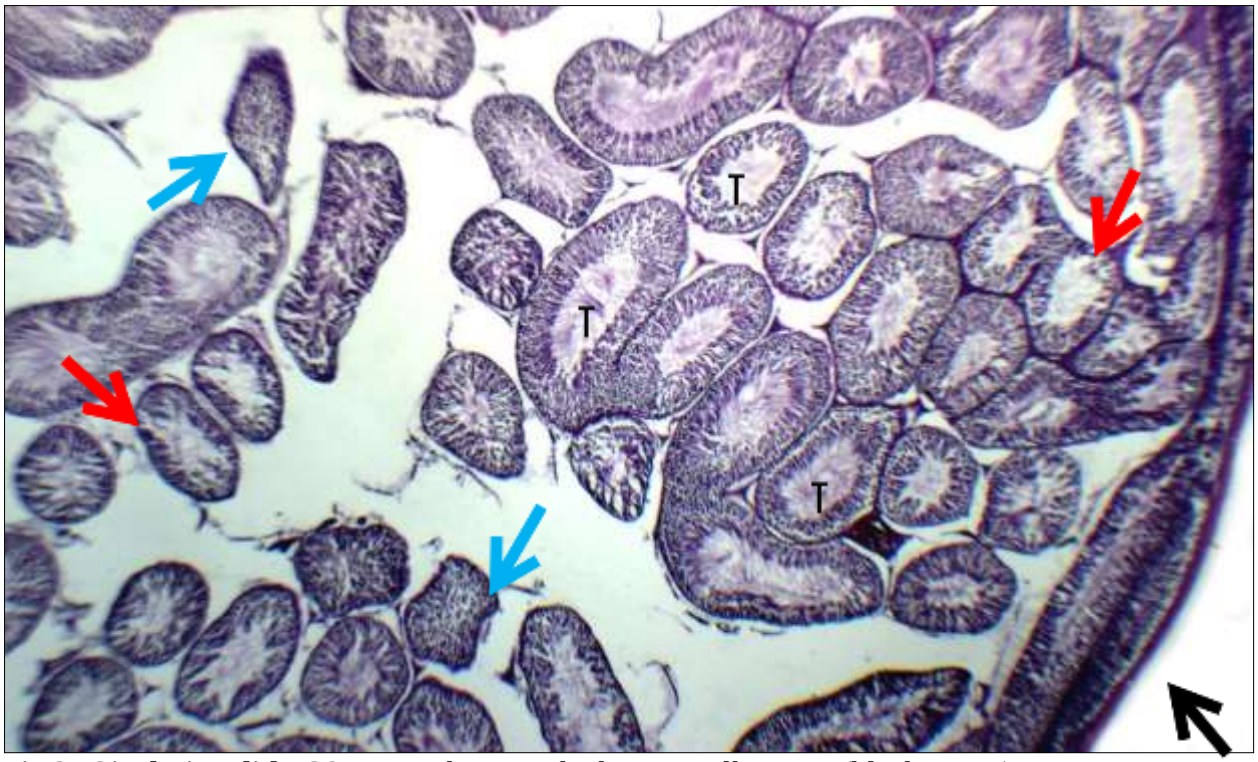

Fig 3: Cisplatin: slide G2: testis showing thick tunica albuginea (black arrow), widely-spaced small-sized distorted tubules (blue arrow) with reduction of germinal lining (red arrow) (H&E X 100)

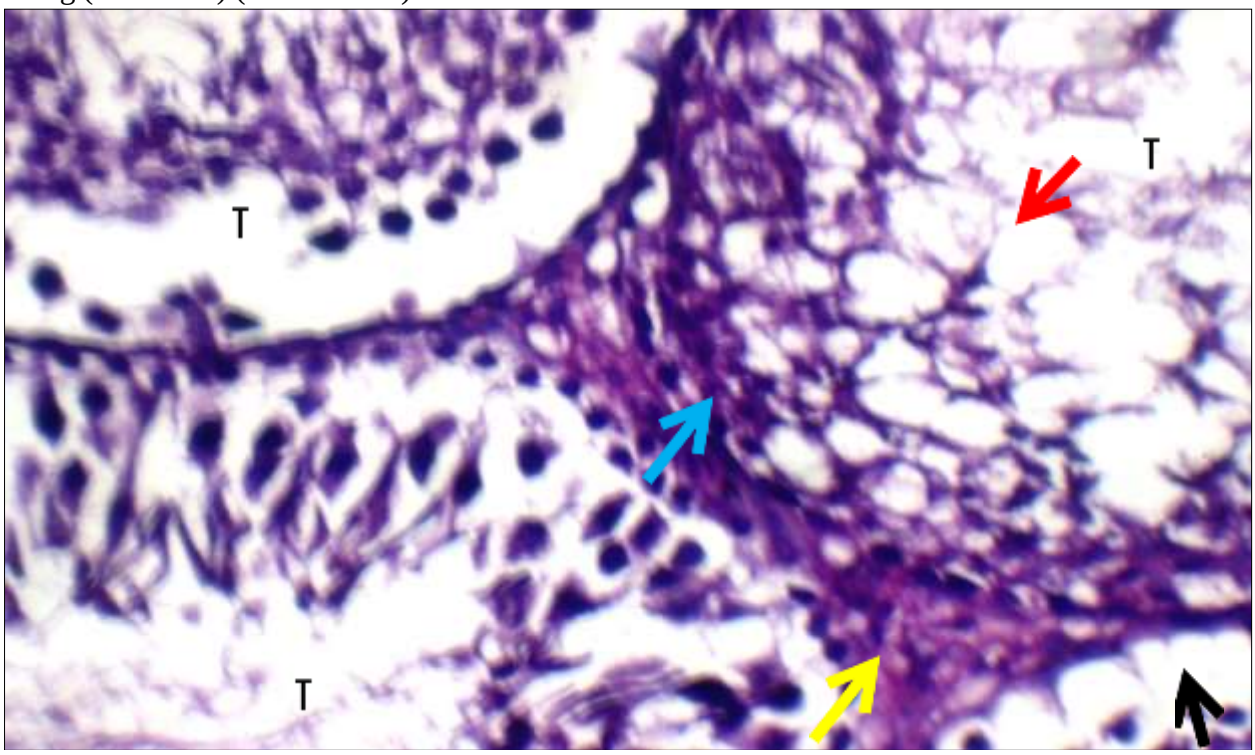

Fig 4: Cisplatin: slide G2: higher power view showing tubules with thick basement membrane (black arrow), marked reduction of germinal lining (blue arrow), no spermatozoa in some tubules (red arrow), and average interstitium showing Leydig cells (yellow arrow) (H&E X 400)

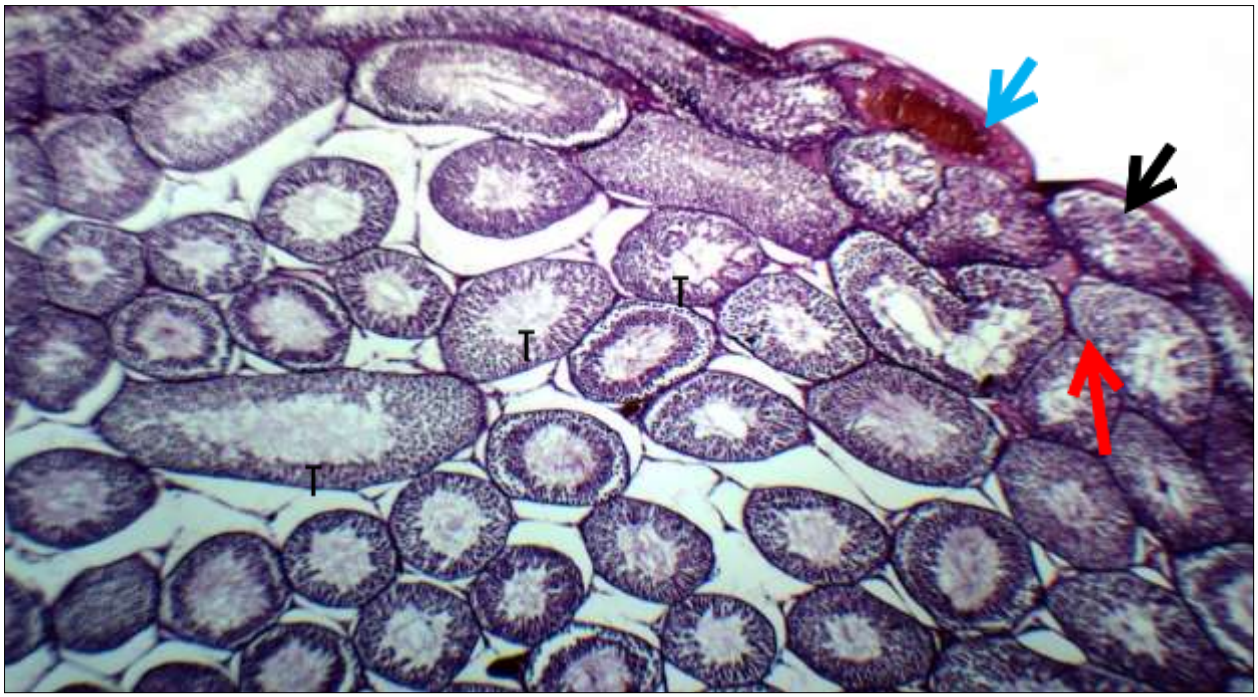

Fig 5: Group 3: slide G3: testis showing average tunica albuginea (black arrow), average sub-capsular blood vessels (blue arrow), average sized tubules (T), and mildly edematous interstitium (red arrow) (H&E X 100)

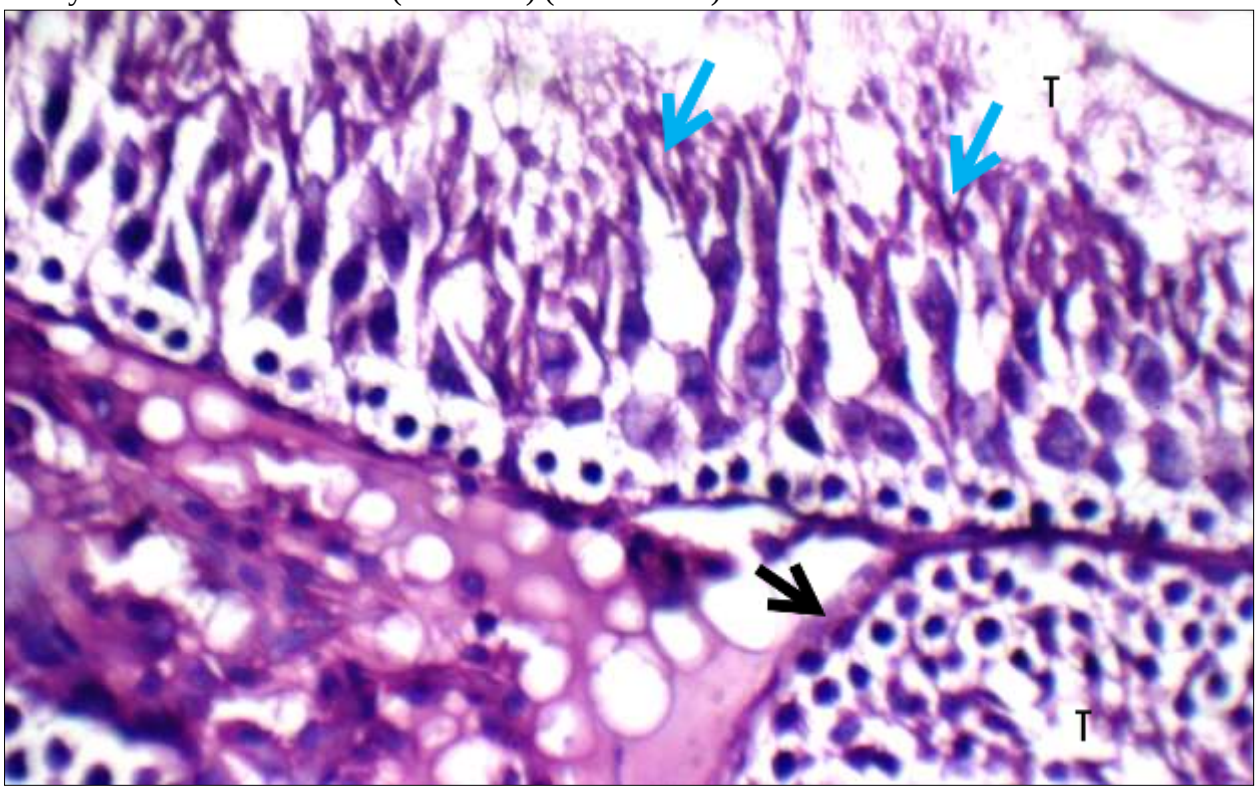

Fig 6: Group 3: slide G3: higher power view showing tubules with mildly thickened basement membrane (black arrow), average germinal lining with full spermatogenesis (blue arrow), and mildly edematous interstitium (red arrow) (H&E X 400)

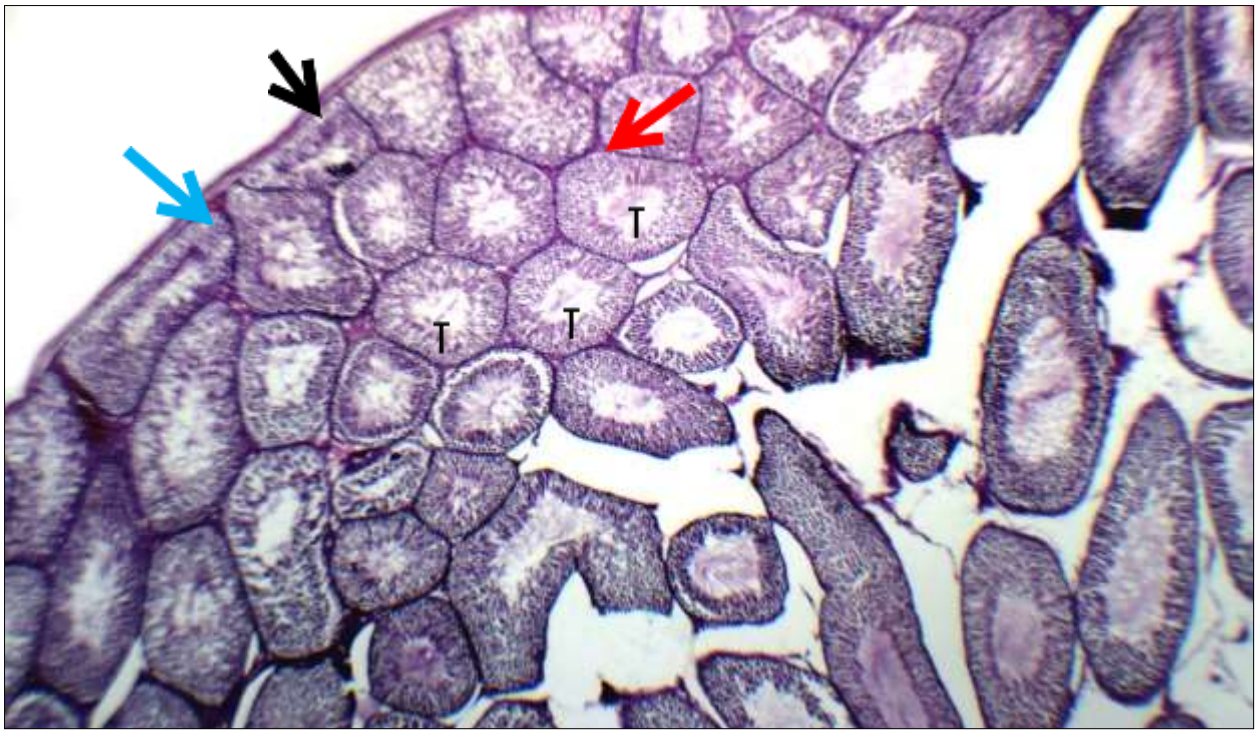

Fig 7: Group 4: slide G4: testis showing average tunica albuginea (black arrow), average sub-capsular blood vessels (blue arrow), average sized tubules (T), and average interstitium (red arrow) (H&E X 100)

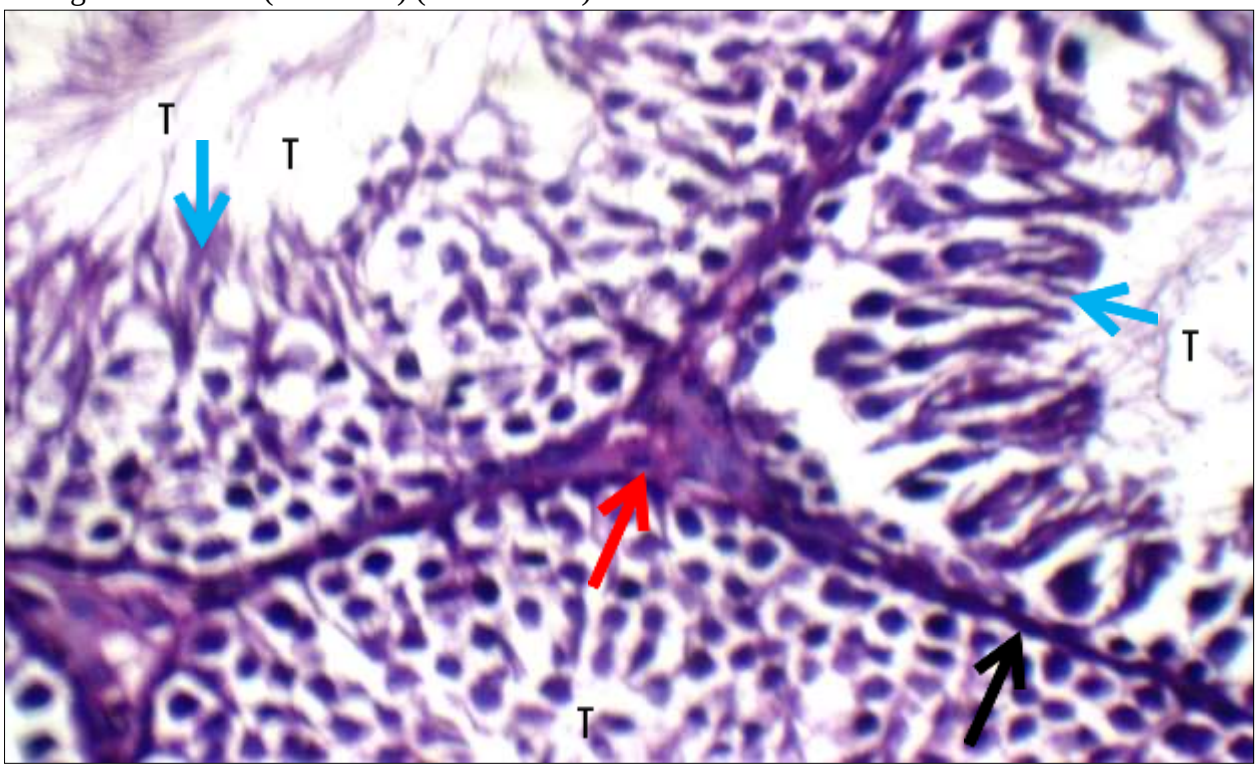

Fig 8: Group 4: slide G4: higher power view showing tubules with average basement membrane (black arrow), average germinal lining with full spermatogenesis (blue arrow), and average interstitium with average Leydig cells (red arrow) (H&E X 400)

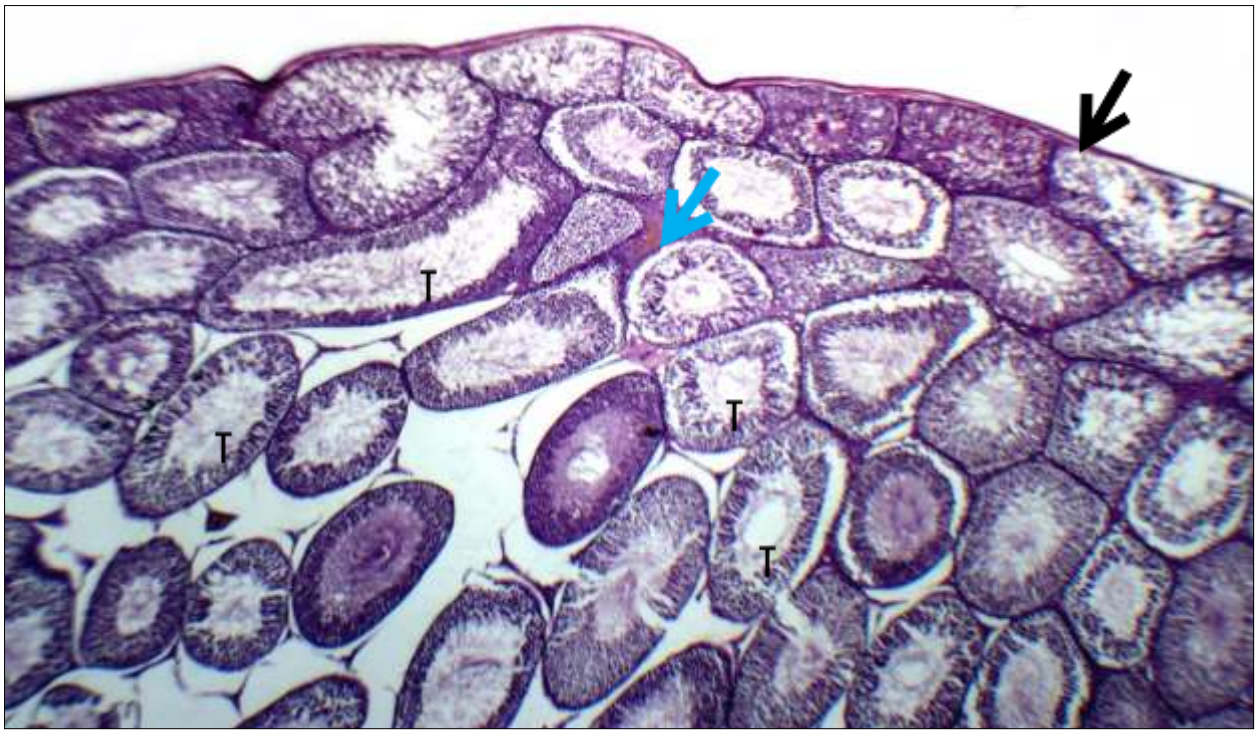

Fig 9: Group 5: slide G5: testis showing average tunica albuginea (black arrow), average sized tubules (T), and mildly edematous interstitium (blue arrow) (H&E X 100)

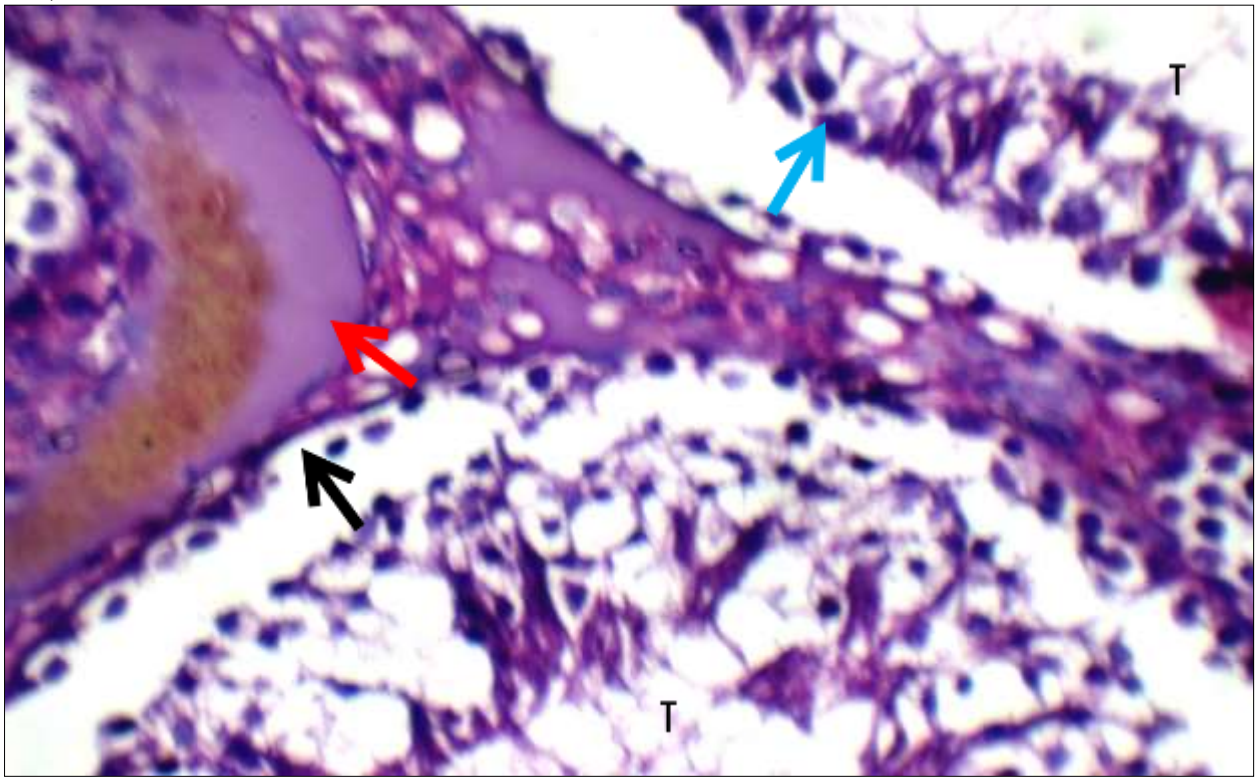

Fig10: Group 5: slide G5: higher power view showing tubules with average basement membrane (black arrow), detached thin germinal lining with reduction of spermatogenesis (blue arrow), and markedly edematous interstitium with dilated thrombosed interstitial blood vessels (red arrow) (H&E X 400)

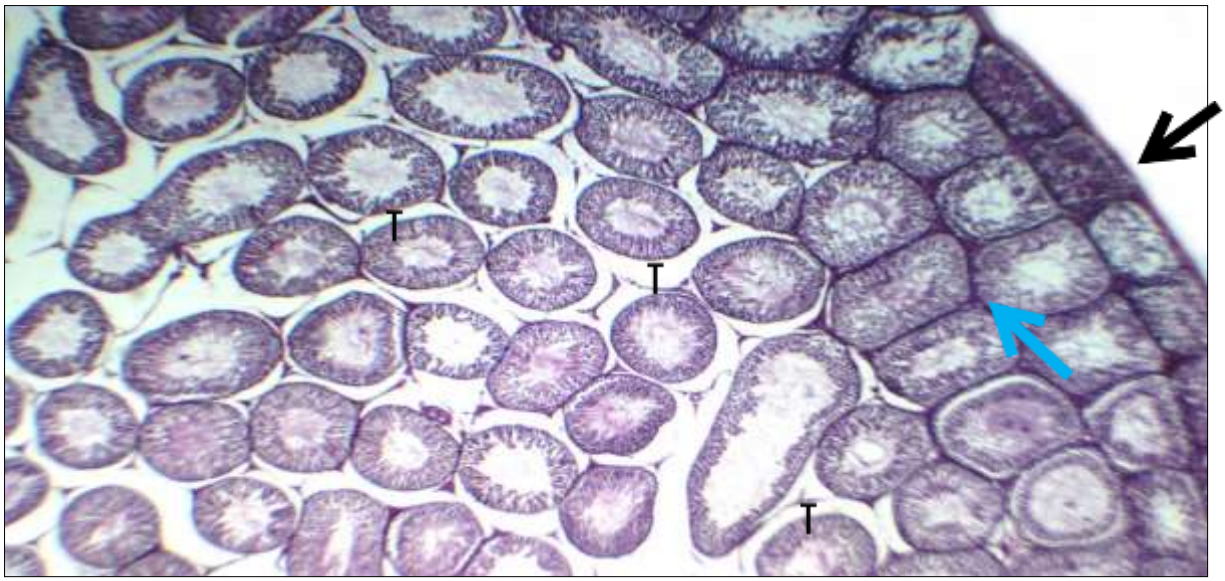

Fig 11: Group 6: slide G6: testis showing average tunica albuginea (black arrow), average sized tubules (T), and average interstitium (blue arrow) (H&E X 100)

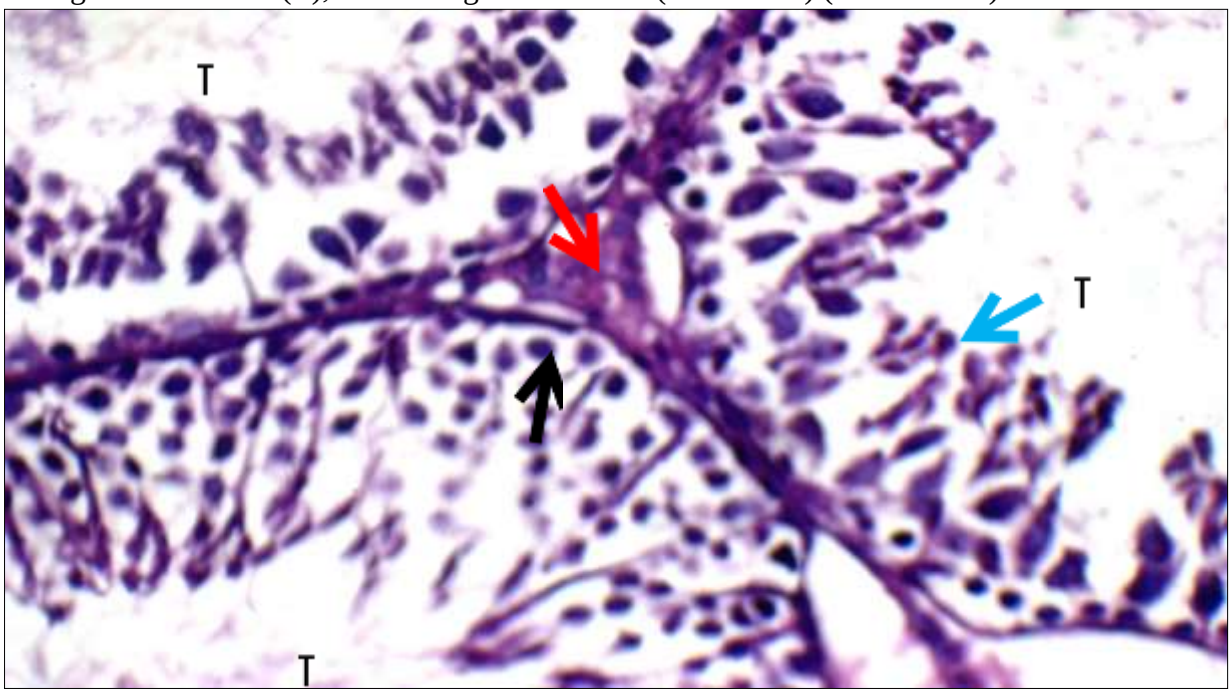

Fig 12: Group 6: slide G6: higher power view showing tubules with average basement membrane (black arrow), average germinal lining with full spermatogenesis (blue arrow), and average interstitium with average Leydig cells (red arrow) (H&E X 400)

CONCLOUSION

Peel and juice of pomegranate contain numerous important ingredients polyphenolic, vitamins and minerals and such ingredients show therapeutics importance to protect fertility in male rats from inducing cisplatin. Thus, it could be confirmed that the consumption of pomegranate is safe and not causes any side effects and also its beneficial effects on health management for both peel and juice.

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تأثير عصير و قشور الرمان على مستوى التستوستيرون ونشاط مضادات الأكسدة في قئران التجارب

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الملخص العربي

اثبتت الدراسات ان الرمان (Punica granatum L.) ذو قيمة حيوية عالية في حمايه النواحي الجنسية لئكور فئران التجارب . لذلك تهدف هذه الدراسة إلى دراسة تأثير عصيروقشور الرمان على مستوى التستوستيرون ونشاط مضادات الأكسدة في قئران التجارب. تم تقدير محتوى الرمان من المعادن والفيتامينات والبوليفينول ومستوي التستوستيرون. أوضحت النتائج أن قشر وعصير الرمان تحتوى على أعلى كمية من المعادن والفيتامينات والمركبات الطبيعية المضادة للأكسدة.

تم تقسيم عدد ستة وثلاثين من الفئران الذكور البالغين إلى ست مجموعات ، ستة فئران لكل مجموعة. تم تغذية الفئران لى نظام غذائي تم تغذية المجموعة الرئيسية الأولى على نظام غذائي أساسي لمدة ٦ أسابيع أخرى واعتبرت الفئران الكنترول السلبي ، وأعطيت المجموعات الخمس الأخرى ٧ مل سيسبلاتين / كجم من وزن الفأر واعتبرت المجموعة الثانيه الكنترول موجب . اما المجموعة الثالثة فتغذت على النظام الغذائي الاساسي مع (٠.٢٥ مل عصير رمان / فأر) بواسطة أنبوب عن طريق الفم (الإعطاء عبر أنبوب). تلقت المجموعة الرابعة على نظام غذائي أساسي و ٠.٧٥ مل عصير رمان / فأر. تلقت المجموعة الخامسة على نظام غذائي أساسي وتلقت ١٠ جرام من مسحوق قشر الرمان المجفف . تلقت المجموعة الأخيرة على نظام غذائي أساسي مع ٢٠ جم من مسحوق قشور الرمان المجفف تم تسجيل زيادة وزن الجسم كل ثلاثة أيام لمدة خمسة أسابيع. علاوة على ذلك ، تم أخذ عينات الدم بسحبها من الضفيرة المدارية وطردها بالطرد المركزي عند ٣٠٠٠ دورة في الدقيقة للحصول على الأمصال بعد ذلك، تم حفظ السيرم في المجمد العميق عند –٢٠ درجة مئوية حتى تحليلها. تم تشريح الخصيه وتجميدها بسرعة في N2 السائل للفحص النسيجي. تم جمع عينات الأنسجة من الاختبارات من الفئران المعالجة والسيطرة بنهاية التجربة.، أظهرت النتائج أن عصير الرمان احتوى على P، Mn، Fe، Cu and بمستويات ٧.٨٢ ، ٥.١٢ ، ٣.٣٧ ، و ٠.٧١ مجم / ١٠٠ جرام على التوالي. أشارت النتائج إلى أن مسحوق قشر الرمان يحتوي على B1 (الثيامين) و B2 (الريبوفلافين) و C (حمض الأسكوربيك) و (α–Tochoferol) E و A (الريتينول) عند

مستويات ٠.١٣١ و ٠.١١ و ١٣.٤ و ٣.٢٥ و ٠.١٥ مجم / ١٠٠ جم مادة جافة ، مقابل ١.٤٢ ، ٠.٩٧ ، ١٥.١٣ ، ١.٧٩ و ٠.٠٩ مجم / ١٠٠ جم مادة جافة لعصير الرمان ، على التوالي. أشارت النتائج إلى أن الإنزيمات المضادة للأكسدة GSH و G-PX و CAT كانت الأقل في مجموعة سيسبلاتين (٢) (١٤.٠٨ مليمول / لتر و ٩.٥٨ مليمول / لتر و ٩.٠٩ ش / مجم على التوالي) والتي يتم حقنها باستخدام سيسبلاتين وتغذية على النظام الغذائي الأساسي من المجموعات الأخرى، فكانت المجموعة الضابطة الموجبه الأعلى في الإنزيمات المضادة للأكسدة ٩٦.٨٨ مليمول / لتر ، ٧٩.١١ مليمول / لتر ، و ١٢.٢٠ ش / مجم على التوالي ، وتغذيت على نظام غذائي أساسي. أظهرت مجموعات سيسبلاتين المختلفة التي تمت معالجتها بعصير الرمان ومسحوق قشر الرمان المجفف أفضل النتائج ، وبالتالي فقد أظهرت مجموعة الفئران (٤) التي تؤخذ عن طريق الفم ١ مل يوميًا ٠.٥٠ مل من عصير الرمان بالإضافة إلى ٠.٥٠ مل ماء مقطر وتغذيت على نظام غذائي أساسي أعطت ٤٣.٦٣ مليمول / لتر ٣٧.٣١ ملي مول / لتر و ٩.٧٢ ش / مجم على التوالي. علاوة على ذلك ، فإن نتائج المجموعة (٦) التي تتغذى على النظام الغذائي الأساسي المستبدل بـ ٢٠ جم من قشر الرمان تعطي ٥٨.٥٩ ملي مول / لتر و ٦٧.١٦ ملي مول / لتر و ١٠.٥٨ ش / مجم ، على التوالي ، مما سبق نستنتج أن أفضل النتائج سجلت عندما تتغذت الفئران على الجرعات الأعلى من قشر الرمان وعصير الرمان٠.٧٥ مل عصير رمان / فأر و ٢٠ وأعطي أفضل النتائج) وتم تأكيد تلك النتائج بالفحص النسيجي للخصية ومما سبق يمكن التوصية بأن عصير الرمان وقشوره مصدرًا جيدًا للصحة الجنسية.

مفتاح الكلمات : عصير الرمان ق،شرالرومان ، الخصوبة ، سيسبلاتين ، الحيوانات المنوية ، الخلايا المنوية.