INTRODUCTION

Renal failure is a condition in which the kidneys fail to remove metabolic end-products from the blood and regulate the fluid, electrolyte, and pH balance of the extracellular fluids. The underlying cause may be renal disease, systemic disease, or urologic defects of non renal origin. Renal failure can occur as an acute or a chronic disorder.**Zhang,**et al.,2013)

Glycerol is sugar alcohol compound. It is a colorless, odorless, viscous liquid that is widely used in pharmaceutical formulations. The glycerol backbone is central to all lipids known as triglycerides. Glycerol is sweet-tasting and generally considered non-toxic. Glycerol-induced Acute Renal Failure is characterized by myoglobinuria,tubular necrosis. ***Karam,et al.,(1995)*** .A standard method of inducing renal failure is byintramuscular administration of 50% glycerol, v/v(8 ml/kg, *im*) .**Savic, et al.,(2002)**

grapefruit (*Citrus ×paradisi*) is a subtropicalcitrus tree known for its sour to semi-sweet fruit, an 18th-century hybrid first bred in Barbados. When found, it was named the "forbidden fruit.**Carrington *,et al.,(2003)*** Grapefruit is an excellent source of many nutrients and phytochemicals that contribute to a healthy diet. ***Fellers, et al.,(1990)***Grapefruit is a good source of vitamin C, contains the fiber pectin, and the pink and red hues contain the beneficial antioxidant lycopene. **Platt (2000).**grapefruit juice ameliorates the nephrotoxicity of amiodarone in albino rats and this may be due to the potent antioxidant effects of its components ***Sakr and El-Gamal, (2016).*** naingin effectively reduced neurotoxicity by attenuating hyperammonemia, suggesting that naringin act as a potential therapeutic agent to treat hyperammonemic rats **Ramakrishnan,et al.,(2016)** Previous

studies identified the potential of GF to favorably affect metabolic syndrome ***Fujioka,et al.,(2006)*** and lipid metabolism ***Goldwasser,et al.,(2010)***. naringin and naringenin, two flavonoids found in high concentrations in grapefruit, may be able to inhibit the development of oral carcinogenesis ***Téleze, et al.,(2010).***Consumption of grapefruit juice was found to be beneficial for human health, including protection against the DNA damage ***Alvarez-Gonalez,-etal.,(2004).*** *Therefore,* the present study is carried out tocompare the protective and therapeutic effects of grapefruit against glycerol induced renal failure.

Materials and Methods

A. Materials:

1. *Grapefruit* :was purchased from alocal market ,Cairo ,Egypt .

2. *glycerol*: was purchased from Ameriya company for Pharmaceutical and Chemical Industries, Cairo , Egypt.

3. *Chemicals and kits* : Kits for biochemical analysis were purchased from the gamma trade company for pharmaceutical and chemicals, Dokki,Egypt. And chemicals were purchased from EL - Gomhorya company cairo ,city ,Egypt .

4. **Animals**: Thirty five mature male albino rats of Sprague Dawley strain weighing (175±10g) and (12–14 weeks old)were purchased from Laboratory of Animal Colony Helwan Egypt.

B. Methods:-

1- Preparation of dried grapefruit:-

Freshgrapefruit was washed with water and cut into slices , removed seeds and dried by the hybrid convective drying system , belonging to the Solar Energy Department,

National Research Center , Dokki , Egypt, at 30- 40 ° C. Thirty kilogram of freshgrapefruit yielded 4.5 kilogram dried grapefruit.

2- Determination of the total phenolic compounds of grapefruit:-

Total phenolic concentration was analyzed using the method described by ***Singleton and Rossi.(1965).***

3- Preparation of the basal diet:

Basal diet was prepared according to **Jia et al.,(1999)**It consists of 20 % protein (casein), 10 % sucrose, 4 % corn oil, 0.2% chlorine chloride, 1% vitamin mixture, 3.5 % salt mixture , 5% fibers (cellulose) and the remainder was corn starch up to 100 % .

4-Induction *of nephrotoxicity.*

Rats injected with a single dose of glycerol (50% v/v glycerol in 0.9% saline at 10 ml/kg) in the last and first days of the experiment according to ***Nobuhito et al.,(1999)***

5-Experimental *Design* :

Rats were maintained under controlled hygienic conditions. Animals were fed on basal diet and water was provided *ad libitum*. Rats were allowed to acclimatize to the laboratory environment for 7 days before starting of the experiment. The experiment was performed on thirty five adult Sprague Dawley rats weighted (175±10g) randomly distributed into 5 groups, of 7 animals each. Group (1) rats were fed on basal diet and served as a negative control, while groups (2) and (3) were kept as a positive control groups intoxicated by a a single dose of glycerol (50% v/v glycerol in 0.9 % saline at 10ml/kg) at the last and first days of the experiment, respectively . Groups (4) and (5) rats were fed on basal diet with 20% of grapefruit (pretreated group) and (treated group) , respectively for 4 weeks.Feed intake was calculated daily and body weight

gain was recorded weekly. At the end of the feeding period, the rats were euthanized by prolonged exposure to ether and blood samples were withdrawn for separating the serum by centrifugation at 8000 rpm for 15 min. Serum samples were kept frozen at -70 Ċ till biochemical analyses. The kidney of sacrificed rats were taken and preserved in 10% formalin solution till processed for the histopathological examination .

6- Kidney function marker:

Blood urea nitrogen was determined using Bio Mérieux kits according to **Patton and Crouch,(1977)** Serum uric acid was determined using the enzymatic colorimetric method as described by ***Fossati,et al.,(1980).*** Serum creatinine concentrations were calorimetrically determined by ***Husdan and Rapoport (1968).*** Serum total protein ,albumin and homocysteine were determined as descrebied by the method of ***Weichselbaum, (1946),Bartholomew and Delaney*****(1966) and*****Ueland,et al.,(1999***) respectively .

Histopathological Examinations :

The Kidney of sacrificed rats were taken and immersed in 10% formalin solution . The fixed specimens were then trimmed , washed and dehydrated in ascending grades of alcohol. They were then cleared in xylol, embedded in paraffin, sectionned at 4-6 microns thickness and stained with Heamtoxylin and Eosin according to***Carleton ,(1979)***

Statistical analysis :

The obtained data were statistically analyzed by using computer .The results were expressed as (mean ± standard deviation "SD") and tested for significance using both one way analysis of variance "ANOVA" test, according to

Armitage ,et al.,1987 and least significant differences "LSD" according to ***Scedecor and Cochran, (1976)***

RESULTS

Data in table (1) showed that, total Polyphenol in grapefruit was 1.40 mg/g.

Table 1 : Total Polyphenols in dry grapefruit.

Parameter	Dry matter of leave of ginkgo biloba g/100 g dw
T. Polyphenols (mg GAE/g)	1.40

GAE : gallic acid equivalents**.**

Data in table (2) showed that ,there were very highly significant **(**P<0.05 **)** ***difference between glycerol*** **(+ve) and control (-ve) group in FI .** Nephrotoxic group (+ve) revealed significant reduction (P<0.05) in final b.wt and BWG% as compared with control (-ve). Pretreated with 20% grapefruit caused a sigficant **(**P<0.05 **)** reduction in final weight and BWG% compared with glycerol (**+ve).While** treated with 20% grapefruit induced improvement in final weight and BWG %. compared with glycerol (**+ve).**

Table (2): Effect of Grapefruit (*Citrus Paradisi* L.) on feed intake, weights and body weight gain% against glycerol induced nephrotoxicity in male rats.

Groups / Parameter	Feed Intake	Initial Weight(g)	Final Weight(g)	BWG%
G(1)-ve control	19.00 ^{a}± 1.00	202.333^{a}±4.041	244.00^{a}±5.291	20.590^{a}±0.523
G(2)+ve Control(pretreated)	18.333^{ab}± 0.577	203.333^{a}±1.154	235.666^{b}±0.577	15.904^{b}±0.839
G(3)+ve control(treated)	14.00 ^{c}± 1.00	202.666^{a}±2.516	212.333 ^{c}± 2.51	4.767 ^{c}± 0.297
G(4)pretreated with 20% grapefruit	16.333 ^{c}± 0.577	202.333^{a}±1.527	221.00^{d}± 1.00	9.228 ^{d}± 0.807
G(5)treated with 20%grapefruit	16.00 ^{b}± 1.00	203.333^{a}±1.154	223.666 ^{b}± 2.88	9.999 ^{b}± 1.228

- Values are expressed as mean ± SD.

- Values at the same column with different letters are significant at P<0.05.

As seen in Table (3) Intraperitoneal injection of a single dose of glycerol to rats at the last and the first days of the experiment caused nephrotoxicity manifested by significant (P < 0.05) decreases in serum levels of total proteins (6.333± 0.152 and 6.100± 0.100, respectively) when compared with the normal (negative) control group(6.900±0.173) . As well as there was a significant (P < 0.05) decrease in serum level of albumin in both control (+) positive (2.333± 0.070 and 3.086± 0.075, respectively) compared to control (-) negative group(3.893±0.090). Pretreatment and treatment with grapefruit to glycerol-injected rats showed significant (P < 0.05) improvement in all tested protein metabolism parameters, compared to both (+ve) groups.

Table (3): Effect of Grapefruit (*Citrus Paradisi* L.) on serum protein and albumin against glycerol induced nephrotoxicity in male rats.

Parameters / Groups	Protein	Albumin
G(1) –ve control	6.900[a]±0.173	3.893[a]±0.090
G(2)+veControl(pretreated)	6.333 [b]± 0.152	2.333 [d]± 0.070
G(3)+ve control(treated)	6.100 [d]± 0.100	3.086 [c]± 0.075
G(4)pretreated with 20% grapefruit	6.700 [a]± 0.100	3.193 [b]± 0.167
G(5)treated with 20%grapefrui	6.533 [b]± 0.153	3.833 [a]± 0.208

- Values are expressed as mean ± SD.
- Values at the same column with different letters are significant P<0.05.

Effect of pretreated and treated of grapefruit on kidney function against GM-induced nephrotoxicity in male rats is shown in table (4). The results revealed that, acute intoxication of rats by glycerol induced significant(P < 0.05) elevation in serum level of urea nitrogen for both control +ve ,(130.666± 6.027,and 56.00± 3.605 , respectively) compared to control (-) negative group(16.530± 1.513). As

well as there was a significant ($P < 0.05$) increase in serum level of creatinine in both control (+) positive (5.410± 0.085and 1.583± 0.144, respectively) compared to control (-) negative group(0.766± 0.05). Fed of grapefruit to glycerol injected rats, showed significant amelioration in all tested kidney function parameters. The grapefruit lowers the elevated serum levels of urea nitrogen and creatinine.There were significant ($p<0.05$) difference between pretreated and treated groups and glycerol (+ve) group . On the other hand, there was a significant ($P < 0.05$) increases in serum level of **homocysteine** in both +ve groups compared to control (-) negative group. Pretreated and treated with grapefruit caused a significant ($P < 0.05$) decreases in serum **homocysteine level compared to** (+ve) group .

Table (4): Effect of Grapefruit (*Citrus Paradisi* L.) on serum urea nitrogen ,creatinine and homocysteine of against glycerol induced nephrotoxicity in male rats.

Parameters / Groups	Urea nitrogen mg/dl	Creatinine mg/dl	Homocysteine
G(1) –ve control	16.530 ^{c}± 1.513	0.766 ^{c}± 0.05	23.533 ^{b}± 1.858
G(2)+ve Control(pretread)	130.666 ^{a}± 6.027	5.410 ^{a}± 0.085	37.500 ^{a}± 1.500
G(3)+ve control(treated)	56.00 ^{a}± 3.605	1.583 ^{a}± 0.144	38.466 ^{a}± 1.747
G(4)pretreated with 20% grapefruit	127.833 $^{a\ b}$± 10.550	3.880 ^{bc}± 0.158	18.200 ^{c}± 1.552
G(5)treated with 20%grapefrui	20.633 $^{b\ c}$± 0.896	1.020 ^{b}± 0.045	21.666 ^{b}± 1.222

- Values are expressed as mean ± SD
- Values at the same column with different letters are significant at $P<0.05$
- CP:Citrus Paradisi

Effect of pretreated and treated of grapefruit on serum levels of ionic sodium , potassium , calcium, and

Phosphorus against Glycerol -induced nephrotoxicity in male rats is shown in table (5) .

Data showed that glycerol(+ve) group had very highly significant($P < 0.05$) elevation in serum levels of **Na , K** and **P** concomitant with significant ($P < 0.05$) reduction in Ca compared to control (-) negative group. Pretreated and treated with grapefruit caused a marked protection evidenced by significant ($P < 0.05$) reduction in serum levels **Na , K** and **P**concomitant with significant ($P < 0.05$) improvement in Ca level compared to (+ve) group . As well as there was a non - significant change in pretreated and treated groups .

Table (5): Effect of Grapefruit (*Citrus Paradisi* L.) on serum levels of sodium , potassium , calcium**, and** Phosphorus **against glycerol induced nephrotoxicity in male rats.**

Parameters / Groups	Na	K	Calcium	Phosphorus
G(1) –ve control	133.333 ^{c}± 2.516	2.530 ^{b}± 0.040	12.300 ^{a}± 0.793	3.153 ^{c}± 0.192
G(2)+ve Control(pretread)	166.00 ^{a}± 2.00	4.010 ^{a}± 0.187	9.300 ^{c}± 0.529	4.663 ^{a}± 0.230
G(3)+ve control(treated)	161.00 ^{a}± 4.582	3.500 ^{a}± 0.222	8.400 ^{d}± 0.360	4.593 ^{a}± 0.110
G(4)pretreated with 20% Grapefruit	149.00 ^{b}± 1.00	3.190 ^{b}± 0.196	11.00 ^{b}± 0.888	3.986 ^{b}± 0.205
G(5)treated with 20%grapefruit	145.00 ^{b}± 2.00	2.643 ^{b}± 0.177	11.566 $^{a\,b}$± 0.305	3.413 $^{b\,c}$± 0.380

- Values are expressed as mean ± SD
- Values at the same column with different letters are significant at $P<0.05$

Histopathological Investigations:

Histological examination of kidneys of healthy rats showed the normal histological structure of renal parenchyma as illustrated in Photo (1). Kidney of rat from

group 2 positive control group (pretreated) vacuolation of epithelial lining renal tubules and presence of eosinophilic protein cast in the lumen of renal tubules Photo.(2). As well as Kidney of rat from group 3 positive control group (treated)showed tubular necrosis and calcification , photo.(3). Examination of kidneys of rats from group pretreated with 20% grapefruit showed no histopathological changes**,** photo.(4)**.Whereas** kidney of rat from **rats fed on** 20% grapefruit showed presence of eosinophilic protein cast in the lumen of renal tubules, photo.(5).

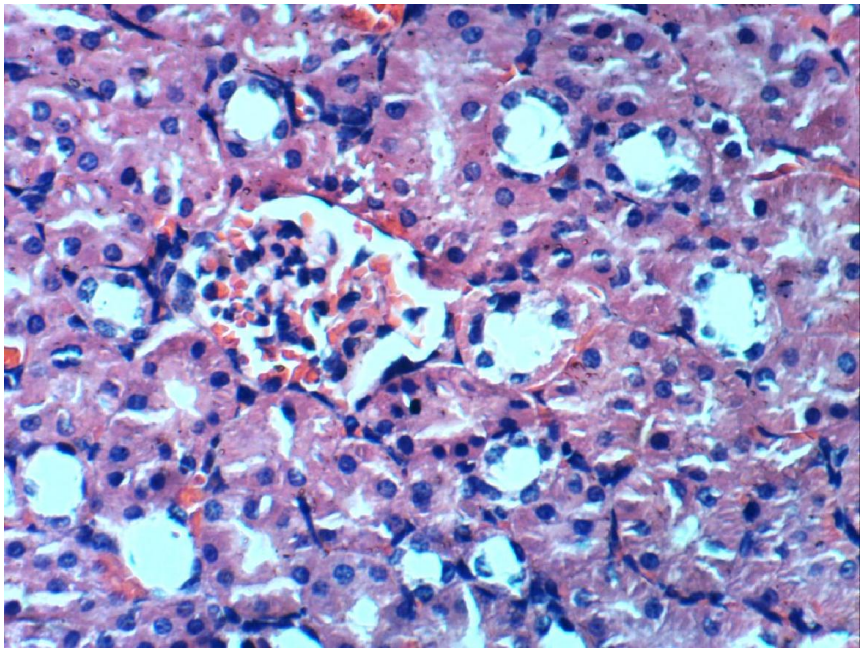

Photo. (1): Kidney of rats from Control negative group showing the normal histological structure of renal parenchyma (H & E X 400)

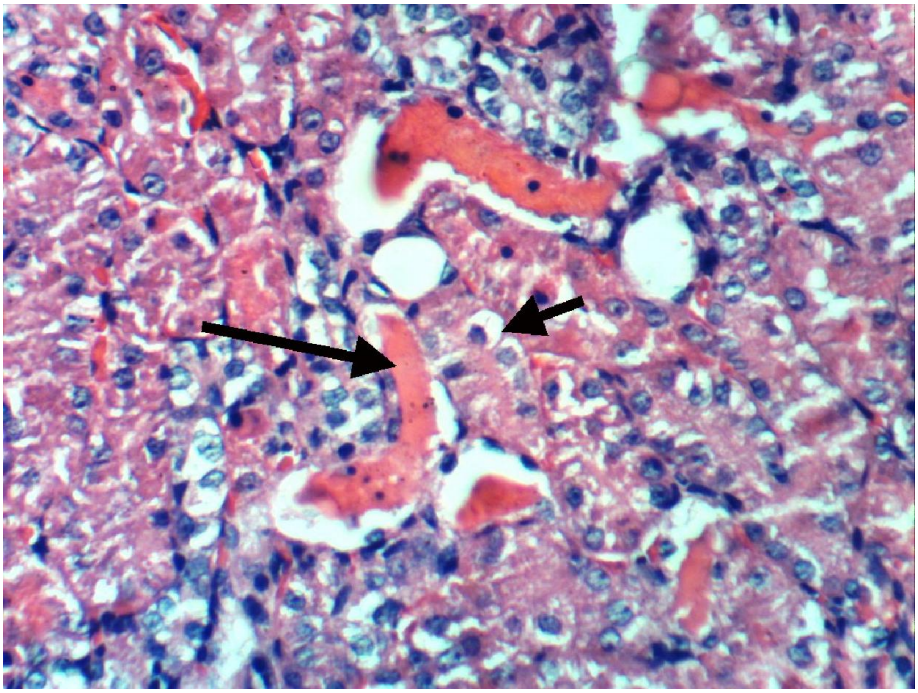

Photo. (2): Kidney of rats from positive pretreated control group showing vacuolation of epithelial lining renal tubules and presence of eosinophilic protein cast in the lumen of renal tubules (H & E X 400).

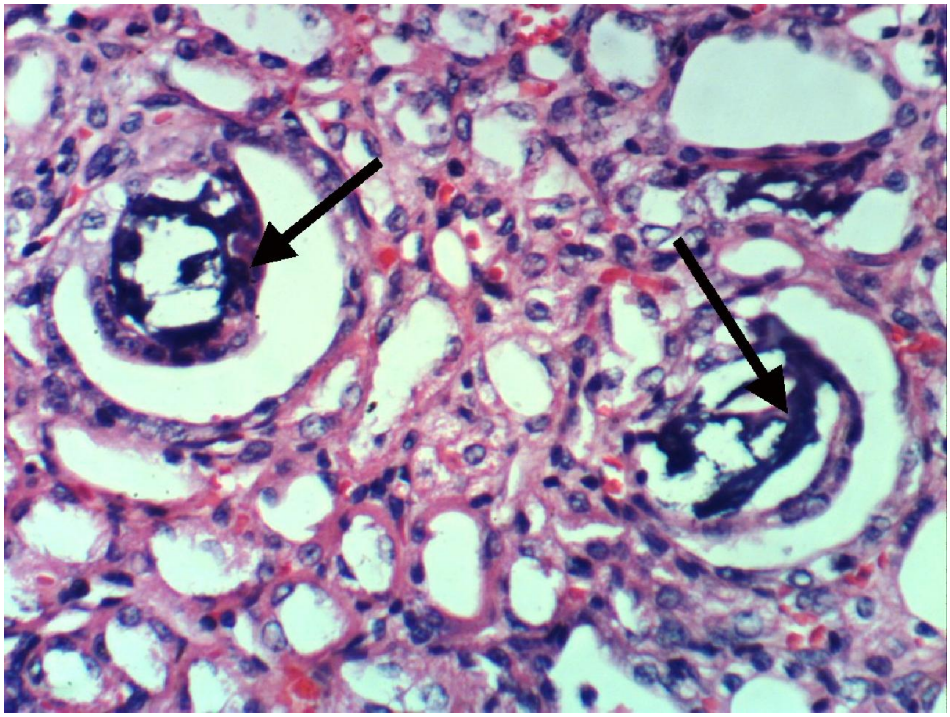

Photo. (3): Kidney of rat from group 3 positive treated control group showing tubular necrosis and calcification (H & E X 400)

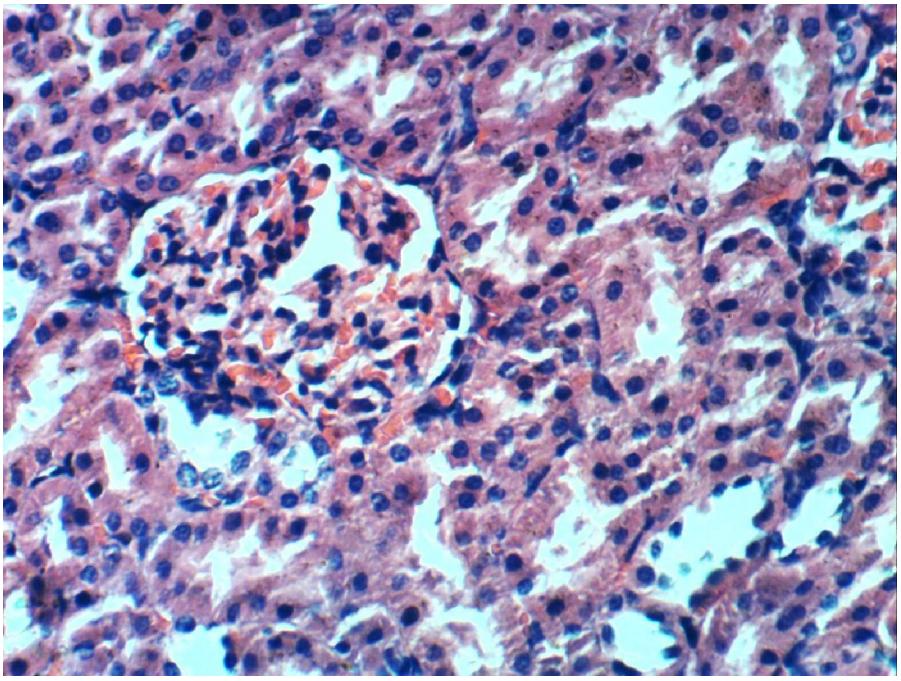

Photo. (4): Kidney of rat from group 4 pretreated with 20% grapefruit showing no histopathological changes (H & E X 400)(preteated)

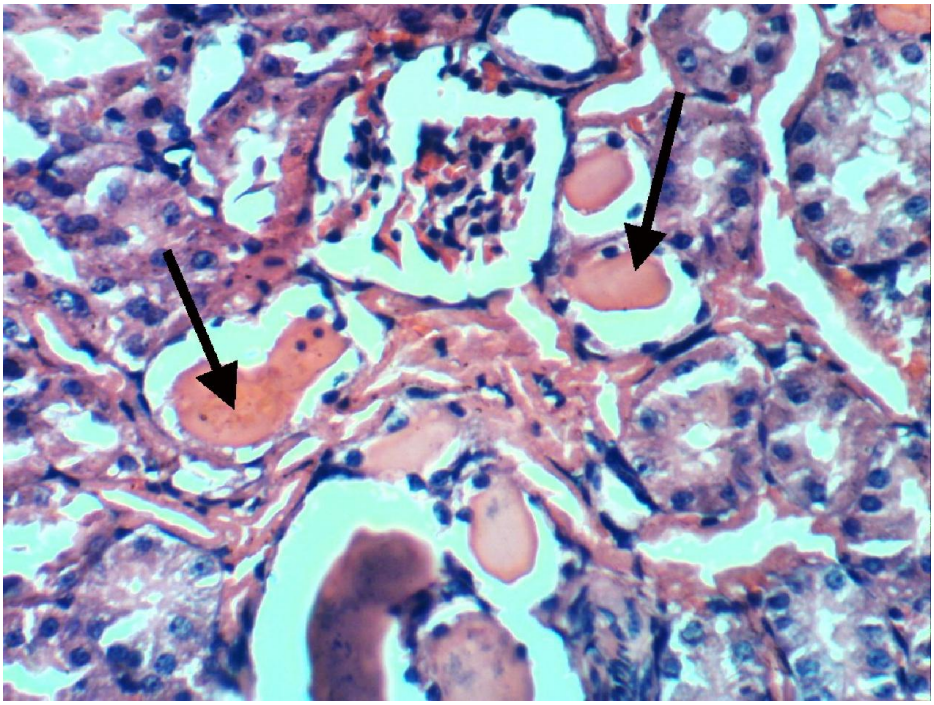

Photo. (5): Kidney of rat from group (5) 20% grapefruit showing presence of eosinophilic protein cast in the lumen of renal tubules(treated).

DISCUSSION

Nephrotoxicity represents a major health problem and accounts for high incidence among population all over the world ***Jain ,et al.,2013*** Glycerol-induced Acute Renal Failure is characterized by myoglobinuria,tubular necrosis ***Karam,et al.,(1999)***.

Many plants contain antioxidant compounds and these compounds protect cells against the damaging effects of reactive oxygen species (ROS) such as single oxygen, superoxide, peroxyl radicals, hydrox radicals and

peroxynitrite . Thus compounds or antioxidants that can scavenge free radicals have vital role in improvement of diseased conditions ***Fouad,et al.,(2014)***

Body weight is the most sensitive indicator of adverse effects of chemical toxicants and xenobiotics. Glycerol induced statistical significant loss of body weight in glycerol groupcompared with control. This result was agree with**Farouk,et al.,(2015)**who mentioned that grapefruit juice significantly produced its weight effect after one week of administration and lasted till the end of the experiment. Furthermore, these results in accordance with those previously reported by **(*Backey*,*et al.,1988, Cerda*, et al.,1988*Kurowska* ,et al., (2000)and*Heidi*,et al.,(2011)** who showed that grapefruit by itself may not have a direct effect on the body weight yet it may help people on diet by delaying gastric emptying, expanding satiety and reducing the feeling of empty stomach . On the other hand these results were disagreed with ***Caitlin, et al.,2013*** who indicated that , consumption of grapefruit daily for 6 weeks does not significantly decrease body weight.

The increase catabolism accompanied by anorexiaand the decrease of feed intake may be the causes of bodyweight loss seen in glycerol injection. On the other hand, pretreated and treated with grapefruit to glycerol-injected rats induced markedamelioration on body weight

as comparing with glycerol (+ve)group. These results agree with **Farouk,et al.,(2015)**who illustrated that grapefruit juice significantly increased food consumption .

In the current study the results revealed that, serum level of total protein and albumin exhibited significant decrease in glycerol (+ve)group as compared with the corresponding values of thecontrol (-ve) group. These results were partially similar to those obtained by **Farouk,et al.,(2015)**who found that grapefruit juice administration either before infection or before treatment with artemether was significantly decreased on serum protein .In addition, decreased albumin is an important predictor of progression of kidney diseases. Pretreatment and treatment with grapefruit showed significant improvementin all tested protein metabolism parameters.These results were in the same line with ***Osfor ,et al.,(2013)***who revealed that the groups of rats which received diets supplemented with 10% and 20% OrAP(orange albedo powder) for six weeks spontaneously were significantly increased on serum albumin.

The present results indicated that, there were significantelevation in urea nitrogen and creatinine in glycerol (+ve) group as compared with control (-ve) group. These results were partially similar to those obtained by ***Moghaddam, et al.,(2010)Ullah, etal.,(2014)*** and ***AL-Yahya ,et al.,(2015)***which used daily dose of 80 mg/kg of gentamicin to produce significant nephrotoxicity. Blood urea nitrogen and serum creatinine increased with significant fall in glomerular filteration, measured by creatinine clearance was observed with gentamicin.

Pretreated and treated with grapefruit to glycerolinjected rats, showed

significant amelioration in all tested kidney functionparameters as compared with the glycerol (+ve) group. Thepresent findings were in the same line with those reported by**Farouk,et al.,(2015)**who mentioned that

grapefruit juice significantly decrease urea nitrogen effect after one week of administration and lasted till the end of the experiment.Moreover, the prevalent antioxidant activity in grapefruit due to its phenolic compounds content, this propertymay contribute in enhancing renal functions viasuppressing oxidative stress which induced tubular injury.

Renal insufficiency is invariably accompanied by elevated plasma concentrations of the sulfur-containing and potentially vasculotoxic amino acid homocysteine. Glycerol induced statistical significant increases in homocysteine level , this finding was supported by ***van Guldener and Stehouwer.,(2005)***who concluded that there is a strong relationship between glomerular filtration rate and plasma homocysteine concentration. Unlike creatinine, however, homocysteine is avidly reabsorbed in the renal tubules, and its urinary excretion is minimal. Also , thepresent findings were in the same line with those reported by ***van Guldener and Robinson., (2000)***who mentioned that In patients with renal failure, hyperhomocysteinemia is a common feature. The mechanisms include reduced renal elimination of homocysteine and impaired nonrenal disposal, possibly because of inhibition of crucial enzymes in the methionine-homocysteine metabolism by the uremic milieu. Absolute or relative deficiencies of folate, vitamin B6, or vitamin B12 may also play a role. Several case-control and prospective studies have now indicated that hyperhomocystenemia is an independent risk factor for atherothrombotic disease in patients with predialysis and end-stage renal disease. Pretreated and treated with grapefruit to glycerolinjected rats, showedsignificant reduction in homocysteine level as compared with the glycerol (+ve) group.

Pretreated and treated with grapefruit markedly preserved the chang in ionic Na+ , K+ , Ca+ and P+. There were significant differences between the glycerol (+ve) group and all groups pretreated and treated with grapefruit . These results were in accordance with ***those previously reported by Ullah et al.,(2014)and Sudha and Venkatalakshmi.,(2012)***.

In this study, the biochemical data were concordant with pathological findings. Examination of kidney sections of glycerol (+ve) group showed vacuolation of epithelial lining renal tubules and presence of eosinophilic protein cast in the lumen of renal tubules , tubular necrosis and calcification .This may be due to glycerol accumulation in renal cortex exhibited extensive proximal tubular necrosis throughout the corticomedullary region characterized by eosinophilic tubules with the remnants of karyolytic nuclei ***Midhun*** et al., (2012)

Pretreated with grapefruit remarkably minimized the structural changes in kidney, thus may be explained by antioxidative activity of grapefruit that may be attributed to its antioxidant constituents.

CONCLUSIONS

The current study demonstrated that the glycerol injection induces nephrotoxicity manifested by serum and urine biochemical changes and histopathological alterations in rats. Grapefruit powder possesses significant nephro-protective and antioxidant effects. It has an ability to prevent and ameliorate the degeneration and tubular necrosis induced by glycerol in the kidney of rats. This is assessed by renal functional and histological examinations.

Reference

- ***AL-Yahya, M. A., Mothana, R. A., AL-Said, M. S., AL-Dosari, M., AL-Sohaibani, M., Parvez, M. K.,and Rafatullah, S.(2015):***protective effect ofcitrus medica 'otroj' extract ongentamicin induced nephrotoxicity andoxidative damage in rat kidney.Digest Journal of Nanomaterials & Biostructures (DJNB);Jan-Mar2015, Vol. 10 Issue 1, p19**.**
- ***Alvarez-Gonalez, E., Madrigal-Bujaidar, L., Martino-Roaro, L., Espinosa-Aguirre, J.J.(2004):*** Antigenotoxic and antioxidant effect of grape fruit juice in mice treated with daunoubicin. Toxicol Lett; 152(3): 203-211.
- ***Armitage,P. and Berry, G.(1987):***Statistical Methods in Medical Reserearch. Blackwell , Oxford,UK, pp.93-213.
- ***Backey, P.A., Cerda, J.J., Burgin, C.W.*** et al. (1988): Grapefruit pectin inhibits hypercholesterolemia and atherosc-lerosis in miniature swine. ClinCardiol., 11: 595–600.
- ***Caitlin, A., Dow, Betsy, C., Wertheim, Bhimanagouda, S., Patil., and Cynthia, A. Thomson***.(2013): Daily Consumption of Grapefruit for 6 Weeks Reduces Urine F2-Isoprostanes in Overweight Adults with High Baseline but Has No Effect on Plasma High-Sensitivity C-Reactive Protein or Soluble Vascular Cellular Adhesion Molecule. J Nutr. Oct; 143(10): Values 1586–1592.
- ***Carleton ,H.(1979):***Histological Technique .4th Ed ., London.
- **Carrington, Sean; Fraser, Henry, C**. (2003): "Grapefruit" . A~Z of Barbados Heritage.Macmillan Caribbean. pp. 90–9.
- ***Cerda, J.J., Robbins, F.L., Burgin, C.W., Baumgartner, T.G.,and Rice, R.W.(September 1988):*** "The effects of grapefruit pectin on patients at risk for coronary heart disease without altering diet or lifestyle". Clin Cardiol 11 (9): 589–94.
- ***Farouk, H.,Sawsan, S., Mahmoud. , Bahia A. El-Sayeh, and Ola A.***(2015): Sharaf.Effect of grapefruit juice and sibutramine on body weight loss in obese rats.African journal of pharmacy and pharmacology.Vol.9(8),pp.265-273,29 February.
- ***Fellers, P.J., Nikdel, S., and Lee, H. S. (August 1990).*** *"Nutrient content and nutrition labeling of several processed Florida citrus juice products". Journal of the American Dietetic Association 90 (8): 1079–84***.**

- ***Fossati, P., Prenciple, L. and Berti, G.(1980):*** Enzymatic colorimetric method for determination of uric acid in serum Clin.Chem.26(2):227-273.
- ***Fouad, A.A.,Albuali, W.H.,Zahran,A.,and Gomaa, W. (2014):***Protective effect of naringenin against gentamicin induced nephrotoxicity in rats Environ. Toxicol Pharmacol. , 38(2):420-429.
- ***Fujioka, K., Greenway, F.,and Sheard, J.(2006):*** The effects of grapefruit on weight and insulin resistance: relationship to the metabolic syndrome. J Med Food 9; 49-54: 19) .
- ***Goldwasser, J., Cohen, P.Y.,and Yang, E.(2010):*** Transcriptional regulation of human and rat hepatic lipid metabolism by the grapefruit flavonoid naringenin: role of PPARα, PPARγ and LXRα. PLoS One. Aug 25;5(8):e12399. (Epub).
- ***Heidi J Silver, Mary S Dietrich, Kevin D Niswender.(2011***): Effects of grapefruit, grapefruit juice and water preloads on energy balance, weight loss, body composition, and cardiometabolic risk in free-living obese adults. Nutrition & Metabolism. 2011 8:8. doi:10.1186/1743-7075-8-8
- ***Husdan, H., and Rapoport, A.(1968):***Estimation of creatinine by the Jaffe reaction. A comparison of three methods. Clin Chem. Mar;14(3):222-238.
- ***Jain , A.,Nahata,A., and Singhai,A.K.(2013):***Effect of Tephrosia Purpurea (L.) leaves on gentamicin induced nephrotoxicity in rats . Sci.Pharm.,81(4):1071-1087.
- ***Jia, Z.S., Tang, M.C., Wu J.M. (1999):*** The determination of flavonoid contents in mulberry and their scavenging effects on superoxide radicals. Food Chem, 64, 555–559.
- ***Karam, H., Bruneval, P., Clozel, J.P., Loffler, B.M., Bariety, J.,and Clozel, M.(1995):***Role of endothelin in ARF due to rhabdomylosis in rats. J PharmacolExpTher, 1995, 274, 481–486.
- ***Kurowska, E.M., Borradaile, N.M., Spence, J.D., Carroll, K.K. 2000.*** Hypocholesterolemic effects of dietary citrus juices in rabbits. Nutr Res., 20: 121–29.
- ***Midhun, C, Korrapati., Brooke, E, Shaner., and Rick, G, Schnellmann.(2012):***Recovery from Glycerol-Induced Acute

Kidney Injury Is Accelerated by Suramin.Apr; 341(1): 126–136. doi: 10.1124/jpet.111.190249PMCID: PMC3310704.

- ***Moghaddam, A.H., Javaheri, M., Nabavi, S.F., Mahdavi, M.R., Nabavi, S .M., and Ebrahimzadeh M.A. (2010).*** Protective role of pleurotus porrigens (angel's wings) against gentamicin-induced nephrotoxicity in mice. Europ. Rev. Med. Pharmacol. Sci., 14: 1011-1014.
- ***Nobuhito , S., Takahiro, O., Nobuo, H.,Tokuzo, M. and Akira, Y.(1999):***Influence of glycerol –induced acute renal failure on the pharmacokinetics of cycloporin in rats.J. Pharma. Pharmacol,51:397-404.
- ***Osfor,M. M. H., Hegazy,A. Maha., Abd El-moaty., Mohammad, A., Elmadbouly, A. M. R., Afify, Amr, S. M.,and Elbahnasawy.*****(2013):**HypoCholesterolemic and Hypoglycemic Effects of Orange Albedo Powder (Citrus Aurantium L.) on Male Albino Rats, International Journal of Nutrition and Food Sciences. Vol. 2, No. 2.
- ***Patton, C.J.,and Crouch, S . R.(1977):*** Enzymatic colorimetric method for determination of urea in serum .Anal . Chem. 49: 464-469.
- **Platt, R. (2000).** "Current concepts in optimum nutrition for cardiovascular disease".PrevCardiol**3** (2): 83–7.
- ***Ramakrishnan, A ., Vijayakumar, N., and Renuka, M.(2016):*** Naringin regulates glutamate-nitric oxide cGMP pathway in ammonium chloride induced neurotoxicity.Biomed Pharmacother. Dec;84:1717-1726.:
- ***Reeves, P., Nielsen , F.,and Fahmy, G.(1993):***Purified diets for laboratory rodent :Final report of the American Institute of Nutrition and Hoc writing committee on the reformulation of the AIN-76 a rodent diet .J. Nutrtr ., 123 :1939-1951.
- ***Sakr, S. A.,and El-Gamal, E. M. (2016):***Effect of grapefruit juice on amiodarone induced nephrotoxicity in albino rats. Toxicol Ind Health. Jan;32(1):68-75. doi: 10.1177/0748233713 498443. Epub 2013 Sep 10.
- ***Savic V, Vlahovic P, Djordjevic V, Mitic-Zlatkovic M, Avramovic V, Stefanovic, V.(2002):*** Nephroprotective effects of pentoxifylline in experimental myoglobinuric acute renal failure. Pathologie Biologie, 50, 599–607.
- ***Scedecor,G. W.,and Cochran, W. G.(1976):***Statistical

Method:6th ed., Lowa State University, Press, Ames, lowa. U. S. A.

- ***Singleton , V.,and Rossi, J. A.(1965)***:Colorimetry of total phenolics with phosphomolybdic- phosphotungstic acid reagents. Am j Enol vitic16:144-158.
- ***Télez, M., Ortiz-Lastra, E., Gonzalez, A.J., Flores, P., Huerta, I., and Ramírez, J.M.(2010):*** Assessment of the genotoxicity of atenolol in human peripheral blood lymphocytes: correlation between chromosomal fragility and content of micronuclei. Mutat Res; 695(1-2): 46-54.
- ***Ueland,P.M., Refsum., H., Stabler, S. Malinow ,M .R., Andersson ,A., and Allen, R. H***. (1993): Total homocysteine in plasma or serum: methods and clinical applications.ClinChem. 1993 Sep;39(9):1764-79.
- ***Ullah, N.,Khan, ,M. A., Khan .T.,and Ahmad ,W[1].***(2014): Nephroprotectives potentials of Citrus aurantium:a prospective pharmacological study on experimentalmodels.Pak J Pharm Sci. May;27(3):505-10.
- ***van Guldener, C., Robinson, K.(2000)*** :Homocysteine and renal disease.Semin Thromb Hemost. ;26(3):313-24.
- ***van Guldener C,and Stehouwer, C. D. (2005):*** Homocysteine and methionine metabolism in renal failure. May;5(2):201-8.
- ***Weichselbaum, T.E. (1946):*** Estimation of serum total protein by Biruet method. Am J Clin Path. 16: 40-48.
- ***Zhang, J.L., Rusinek, H.,Chandarana, h. and Lee, V.S.(2013):***Functional MRI of the kidneys . kidney Int.:37(2):282-92 .