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ALLEVIATION THE DIMETHOATE NEPHROTOXICITIY USING WATERMELON RINDS TREATMENTS IN MALE ALBINO RATS

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ABSTRACT

The present study was carried out to highlight the protective effect of watermelon rind against dimethoate induced nephrotoxicity. Proximate analysis of watermelon rind in dry weight and some phytochemical screening of watermelon rind, water extract (WE) and ethanolic extract (EE) were determined. The results showed that moisture content, ash, crude protein, crude fat and crude fiber were 10.61, 13.09, 11.17, 2.44 and 17.28% respectively. Also phenolic, flavonoid compounds, tannins and vitamin C were 14.88 mg/g, 2.55 mg/g, 2.11 mg/100g and 6.65 mg/100g respectively in water extract and 13.66 mg/g, 2.34 mg/g, 1.52 mg/100g and 5.22 mg/100g respectively in ethanolic extract. Uric Acid, urea and creatinine were measured in serum. The biochemical analysis showed highly significant ($p<0.05$) increase in uric acid , urea and creatinine levels in rats treated with dimethoate 1/10 and 1/20 LD_{50} compared to control, while the intoxicated groups were treated with WE, EE and watermelon rind powder 5% showed significant improvements for all previously mentioned parameters. These results are confermed by histopathological examination of kidneys.

Key words: dimethoate, renal function ,watermelon rind **.**

INTRODUCTION

Watermelon (*Citrullus lanatus*) is an ancient fruit of great medical interest and rich source of antioxidants. Watermelon is used amazingly for it nutritional and medicinal values because of its high water content which rich in sugar and energy booster, which hydrate body in the case of dehydration, especially during the hot seasons (**Ayoola *et al*., 2012**).

Watermelon rind low in calories and contains 93.4% water, 0.5% protein, 5.3% carbohydrate, fat 0.1%, fiber 0.2%, ash 0.5% and vitamins (A , B and C). In addition, it contains amino acid citrullin ($C_6H_{13}N_3O_3$), aminoacetic acid, malic acid, phosphoric acid, arginine, betaine, lycopene ($C_{40}H_{56}$), carotene, bromine, sodium, potassium, lysine, fructose, dextrose and sucrose. Citrulline and arginine play a role in the formation of urea by liver from ammonia and CO_2 that increases urine. High content of potassium which can help the heart and normalize blood pressure. Lycopene is an antioxidant that is superior to vitamin C and E (**Wanita, 2011**).

Watermelon possessed high level of antioxidants (phytochemical property) which decreases the risk of kidney stone and bone lose due to old age, and it is a powerful diuretic diet, has the availability of amino acids and beta – carotene which protect heart disease. Also rich in lycopene, which is a pigment that gives the red color that naturally, occurred in *Citrulus lanatus* which prevent ailments of prostate and oral cancer. It is a good source of vitamins such as A, B, C, and thiamine. The seeds of the watermelon contained considerable amount of minerals such as calcium, iron, manganese, phosphorus, potassium, sodium, zinc, copper and magnesium that assist in the growth and development of the healthy body which take part in metabolic activities of all living organisms (**Ayoola and Adeyeye, 2011**).

Dimethoate (*O,O*-dimethyl *S*-*N*-methyl carbomyl methyl phosphorodithioate) (DM) is one of the most important organophosphorus insecticide (OPI) used extensively on a large number of crops against several pests. The residue of DM and its analog were found in number of foods including cow's milk (**Srivastava and Raizada, 1996**).

Oral administration of dimethoate in rats induced a marked renal failure characterized by a significant increase in serum creatinine and urea levels.Interestingly,these drastic modifications were accompanied

by a marked enhancement of lipid peroxidation in kidney, indicating a significant induction of oxidative damage and dysfunctions of enzymatic antioxidant defenses. These biochemical alterations were also accompanied by histological changes in kidney revealed by a narrowed Bowman's space, tubular degeneration, tubular cell desquamation, and tubular dilatation of proximal tubules (**Salah *et al*., 2012**). A reduction in creatinine level has occurred after consuming a significant amount of watermelon and the consequent increase in antioxidants level in his blood as well as the subsequent effect of these compounds on kidney function (**Hasanvand *et al*., 2018**).

This study aim to investigate the protective effect of watermelon rind against dimethoate induced nephrotoxicity.

MATERIALS AND METHODS

Materials

Source of sample :

Fresh watermelon was collected from El Obour market in July 2014 Cairo, Egypt.

Chemicals:

All chemicals and reagents were obtained from Sigma chemical Co. (London, Lab. Poole), England (Cairo branch). Kits were obtained from Biodiagnostic Co.

Methods :

Proximate analyses :

The moisture, ash, crude protein, crude fat and crude fiber contents were determined according to **A.O.A.C (2010)**. But carbohydrate was determined by difference.

Preparation of watermelon rind water and ethanolic extracts :

Water and ethanolic extracts of dried watermelon rind powder were prepared according to **Hannah and Krishnakumari (2015)**

Phytochemical analysis :

Tannins were determined as described by **Price *et al*. (1978).** Total phenolics were estimated based on the methods described by **Wolfe *et al*. (2003)**. Gallic acid was used as a standard and total phenolic content were calculated and expressed as mg galic acid

equivalent (GAE) per g sample**.** Flavonoids content was determined using the method of **Jia *et al*. (1999)**. Quercetin was used as a standard and total flavonoid contents were calculated and expressed as mg quercetin equivalent (QE) per g of sample. Vitamin C was determined according to the method of **Ranganna (2004)**. The direct titration method is based on measurement of the extent to which a 2, 6-dicholorophenol-indophenol solution is decolorized by ascorbic acid in sample extracts and in standard ascorbic acid solutions.

Experimental animals :

Forty eight male albino Spargue-Dawely rats weighing 180 ± 20 g. were provided from the animal house of the National Research Centre, Dokki-Giza, Egypt. They were raised in the animal house of pesticide Department, Faculty of Agriculture, Cairo University. The animals were housed in polyethylene cages and maintained at 25 ± 2 °C, relative humidity of 50-60% and 12/12 h light/dark cycle. The animals were adapted on free access of water and fed on basal diet which prepared according to the national research council (1987) for two weeks before initiation of the experiment.

Experimental design :

After two weeks of adaptation, 48 of the male rats were divided into 12 groups (4 rats each) as follows:

Group (1) (Normal Control): Rats were fed on the normal basal diet which used as normal healthy control.

Group (2): Rats were fed on the normal basal diet and ingested orally with dimethoate (DM) (40mg/kg b.w.) about 1/10 LD_{50} as subchronic doses every 2 consecutive days.

Group (3): Rats were fed on the normal basal diet and ingested orally with DM (20mg/kg b.w.) about 1/20 LD 50 as subchronic doses every 2 consecutive days.

Group (4, 5): Rats were fed on the normal basal diet and ingested orally with water (WE) and ethanolic extracts (EE) of watermelon rind (2g /kg b.w. as doses every 2 consecutive days, respectively).

Group (6): Rats were fed on the semi-modified diet (basal diet supplemented with 5% watermelon rind powder).

Group (7): Rats were fed on the normal basal diet, ingested orally with WE (2g/kg b.w.) and DM (40mg/kg b.w.) as subchronic doses every 2 consecutive days, respectively.

Group (8): Rats were fed on the normal basal diet, ingested orally with EE (2g/kg b.w.) and DM (40mg/kg b.w.) as subchronic doses every 2 consecutive days, respectively.

Group (9): Rats were fed on the semi- modified diet like group 6 and ingested orally with DM (40mg/kg b.w.) as subchronic doses every 2 consecutive days.

Group (10): Rats were fed on the normal basal diet, ingested orally with WE (2g/kg b.w.) and DM (20mg/kg b.w.) as subchronic doses every 2 consecutive days, respectively.

Group (11): Rats were fed on the normal basal diet, ingested orally with EE (2g/kg b.w.) and DM (20mg/kg b.w.) as subchronic doses every 2 consecutive days, respectively.

Group (12) rats were fed on the semi- modified diet like group 6 and ingested orally with DM (20mg/kg b.w.) as subchronic doses every 2 consecutive days.

At the end of the experiment (6 weeks), rats were fasted overnight, then decapitated and blood samples of each group were collected for separating serum. Kidney was taken and immersed in 10% formalin solution for the histopatholol gical examination.

Biochemical analysis

Serum blood urea was measured according to the method described by **Fawcett and Scott (1960).** Serum creatinine was analyzed according to **Houot (1985).** Enzymatic determination of uric acid was carried out according to the method described by **Barham and Trinder (1972).**

Histopathological examination

Kidney of the sacrificed rats was taken and immersed in 10% formalion solution, the specimens were then trimmed, washed and dehydrated in ascending grades of alcohol. Dehydrated specimens were cleared in xylol , embedded in paraffin, sectioned at 4-6 microns thickness and stained with heamtoxylin and Eosin for histopathologicl examination according to the method described by **Carleton (1980).**

Statistical analysis

Statistical analysis was carried out according to **Fisher (1970).** LSD (Least significant difference) was used to compare the significant differences between means of treatment as described by **Waller and Duncan (1969).**

RESULTS AND DISCUSSION

Chemical composition and phytochemical analysis of *Citrullus Lanatus*

Chemical composition of dried watermelon rind sample is presented in **Fig. (1).** Data showed that dried watermelon rind contains 14.80 % moisture, 16.50% ash, 15.3% crude protein, 1.8% crude Fat, 13.60% crude fiber and 38.20% total hydrolysable carbohydrate. These results were paralleled with the results established by **Al-Sayed and Ahmed (2013)** who found that watermelon rind moisture content, ash, crude protein, crude fat and crude fiber were 10.61, 13.09, 11.17, 2.44 and 17.28% respectively.

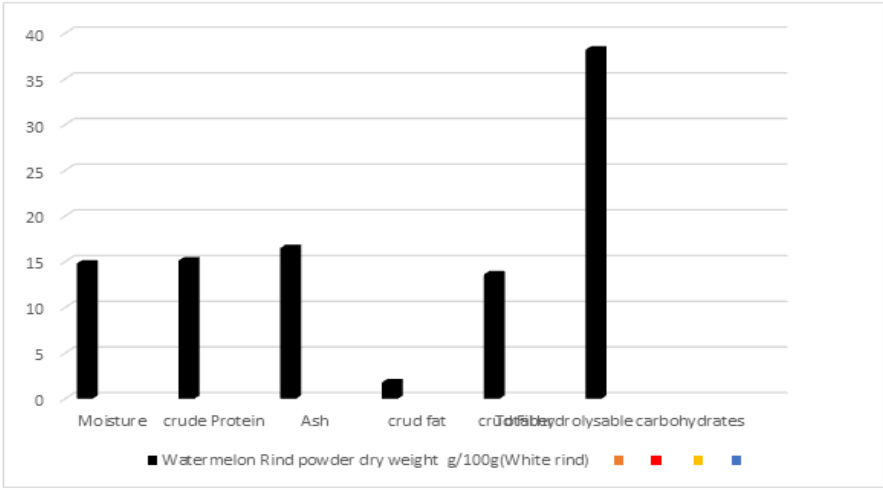

Fig. 1. Chemical composition of dried watermelon rind.

As shown in **Fig (2)** total phenolic contents of water and ethanolic extracts were 14.88 and 13.66 mg/g dry weight, respectivly. Total flavonoid contents of water extract was 2.55 but was 2.34 mg/g dry weight in ethanolic extract.

Tannins content in the water extract was 2.11 mg/100g dry weight but in the ethanolic extract was 1.52 mg/100g. Watermelon rind extracts (water, ethanol) contain high amount of vitamin C which 6.65 mg/100g dry weight in the water extract and was 5.22 mg/100g dry weight in the ethnolic extract.

Rolim *et al.* (2018) evaluated phenolic compounds and antioxidants activities. Total phenolic compounds were found in hydroethanolic and aqueous extracts, especially for melon peel which

amounted 1.016 mg/100g gallic acid equivalent. Flavonoids were found in melon peel aqueous extract which was 262 mg of catechin equivalent (CA)/ 100 g as total flavonoid. In all extracts of melon peel significant amounts of gallic acid, catechin, and eugenol were detected. For total antioxidant capacity, reported as ascorbic acid equivalent, the aqueous and hydroethanolic extracts in peel were 28% and 30%, respectively.

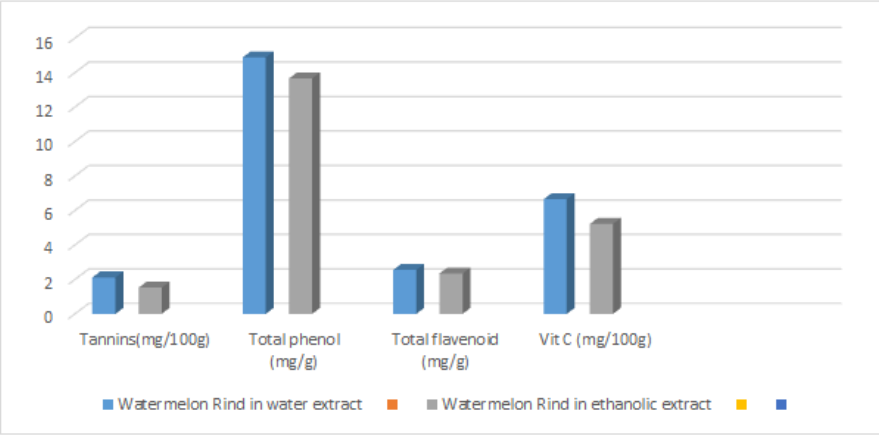

Fig. 2. phytochemical analysis of watermelon rind in water and ethanolic extracts.

Kidneys function of experimental animals :

Fig (3, 4 and 5) showed some effects of the high dose of dimethoate (1/10 LD_{50}) ingestion on the renal function of adult male albino rats which caused a highly significant increases in urea and uric acid (60.88 and 7.68 mg/dL, respectivly) relative to those of normal control (36.85 and 4.23 mg/dL, respectively).Creatinine was increased more than two fold of the value as that of normal control which were 2.16 and 0.80 mg/dL, respectively.

But the effect of the low dose of dimethoate (1/20 LD_{50}) caused a slight significant increases in urea and uric acid (44.14 and 5.82 mg/dL, respectivly) relative to normal control. Also creatinine was increased compare with the normal control (1.34 and 0.80 mg/dL, respectively)

The effect of administration of WE, EE and watermelon rind powder 5% into DM-intoxicated rats (1/10 LD_{50}) caused a highly significant decreases in the level of urea (48.51, 53.44 and 42.87 mg/dL, respectively), a slightly decrease in uric acid (6.19, 6.66 and 6.82 mg/dL, respectively), and creatinine (0.95, 1.09 and 0.96

mg/dL,respectively) relative to those of DM-intoxicated control rats (1/10 LD_{50}). It means that treatments of watermelon rind extract into DM- intoxicated rats improved this disturbance in the kidneys function parameter .

The effect of treatments of WE, EE and watermelon rind powder 5% into DM-intoxicated rats (1/20 LD_{50}) caused an improvement in the level of urea, uric acid and creatinine (kidneys function) .

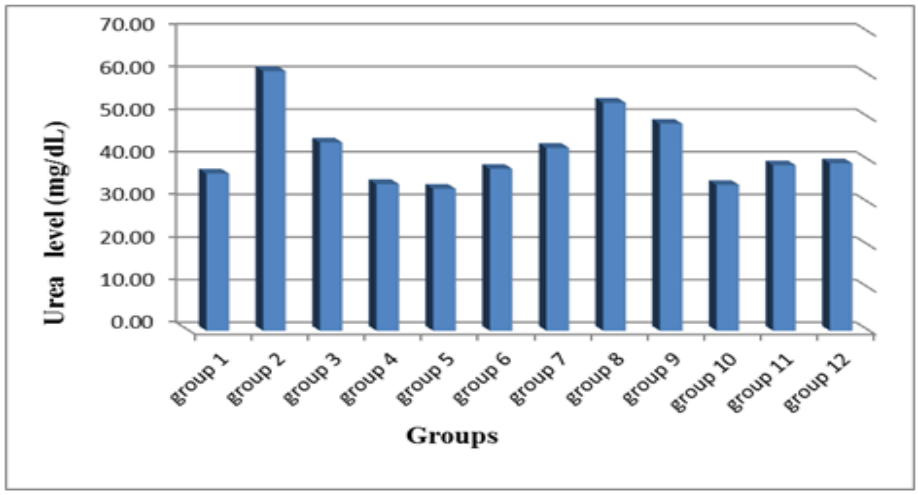

Fig .3. Serum urea (mg/dL) in normal and dimethoate-intoxicated rats (1/10 and 1/20 LD_{50}) treated with WE, EE and watermelon rind powder 5%.

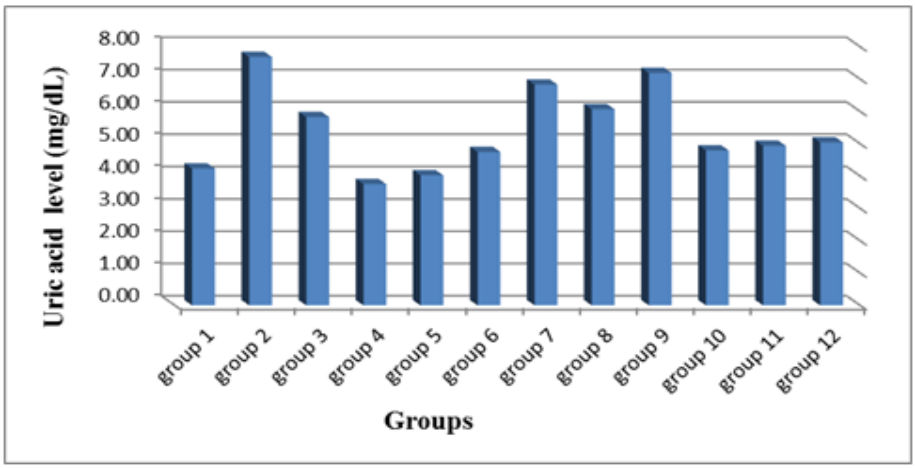

Fig .4. Serum uric acid (mg/dL) in normal and dimethoate-intoxicated rats (1/10 and 1/20 LD_{50}) treated with WE, EE or watermelon rind powder 5%.

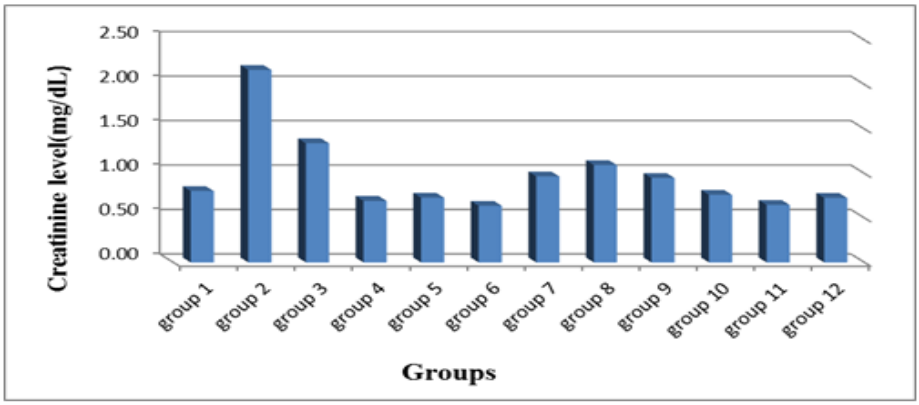

Fig .5. Serum creatinine level (mg/dL) in normal and dimethoate-intoxicated rats (1/10 and 1/20 LD_{50}) treated with WE, EE or watermelon rind powder 5%.

These results are in agreement with **Ben Amara *et al*. (2013)** observation who found that dimethoate DM induced kidney injury which alleviated by natural antioxidants and showed that plasma creatinine and uric acid, kidneys MDA, H_2O_2 levels were higher in the DM group than normal controls. Also, these results agreed the **Salih (2010)** who showed that the uric acid and creatinine levels significantly increased in the serum of dimethoate and diazinon treated rabbits, compared to normal control.

Histopathological examination of kidneys :

Microscopic analysis showed that , kidneys of rat from group 1 revealed the normal histological structure of renal parenchyma **(Fig. 6:a).** Apparent normal renal parenchyma was noticed in kidneys from group 4 **(Fig. 6: b)**. However, slight congestion of glomerular tuft was the only change observed in kidneys from group 5 **(Fig. 6: c)**. No histopathological changes were noticed in kidney of rat from group 6 **(Fig. 6: d)**.Meanwhile, kidneys of rat from group 2 showed necrobiosis of epithelial lining renal tubules and congestion of glomerular tufts **(Fig. 7: a)**. Examined sections from group 7 revealed presence of proteinaceous materials in the lumen of renal tubules and vacuolations of endothelial lining glomerular tuft **(Fig.7:b)**. Moreover, kidneys of rat from group 8 revealed vacuolation of epithelial lining renal tubules **(Figs. 7: c)**. However, kidneys from group 9 showed no histopathological changes **(Fig. 7: d)**. Moreover, kidneys of rat from group 3 showed necrobiosis of epithelial lining renal tubules, proteinaceous material in the Bowman's space **(Fig. 8:a)**. Kidneys of rats from group 10 showed proteinaceous materials in the lumen of renal tubules and Bowman's space **(Fig. 8: b)**. Sections from groups 11 revealed congestion of glomerular tuft and intertubular blood capillaries **(Figs. 8: c)**. Some sections from group 12 showed slight congestion of glomerular tufts **(Fig. 8: d)**.

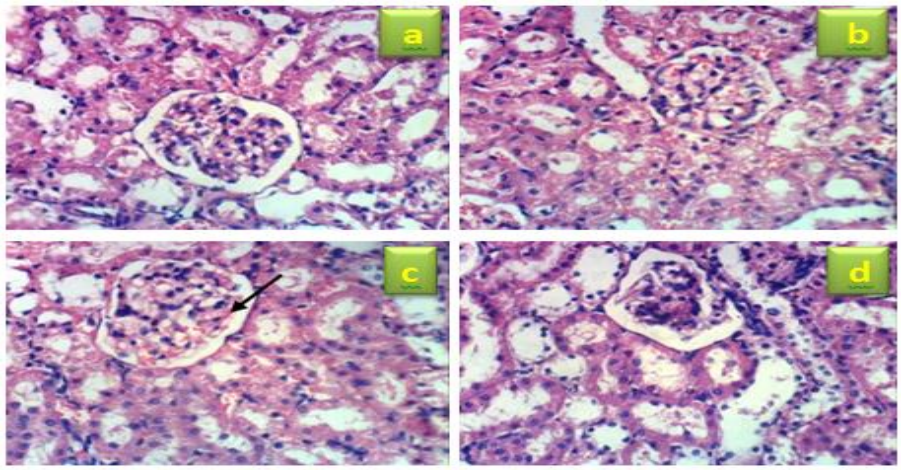

Fig.6. Photomicrographs of kidney: (a) normal control (G1) rats treated with basel diet. (b) rats treated with WE and basel diet as negative control (G4). (c) rats treated with EE and basel diet as negative control (G5) .(d) rats treated with WMRP5% and basel diet as negative control (G6) (H & E X 400).

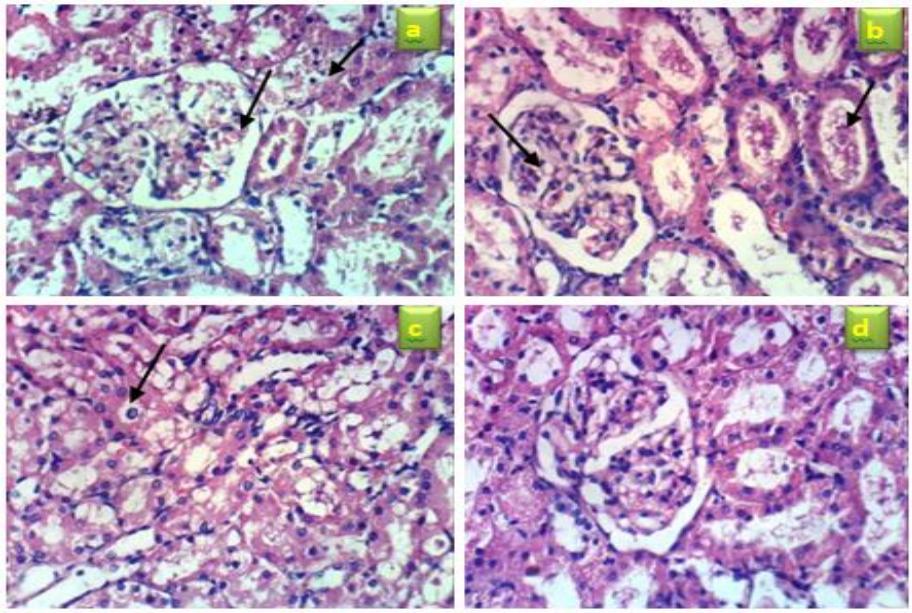

Fig.7. Photomicrographs of kidney: (a) positive control (G2) rats rteated with 1/10 LD50 DM.(b) intoxicated rats treated with 1/10 LD50 DM and WE(G7). (c) intoxicated rats treated with 1/10 LD50 DM and EE (G8).(d) intoxicated rats treated with 1/10 LD50 DM and WMRP5% (G9) (H&E X400).

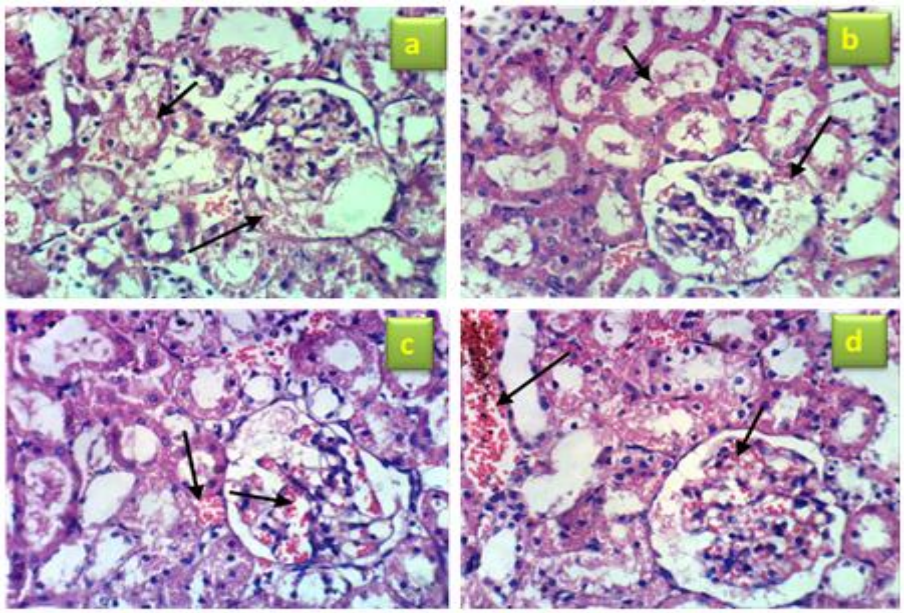

Fig. 8. Photomicrographs of kidney:(a) positive control (G3) rats treated with 1/20LD50 DM . (b) intoxicated rats treated with 1/20 LD50 DM and WE (G10).(c) intoxicated rats treated with 1/20 LD50 DM and EE(G11). (d) intoxicated rats treated with 1/20 LD50 DM and WMRP5% (G12) (H&E X400).

Kidneys dysfunction in DM-ingested rats was substantiated by histopathological data. There was a leucocytic infiltration considered as a prominent response of the body tissue facing any injurious impacts. Renal lesions were also characterized by a vascular congestion and a tubular vacuole formation, indicating the beginning of a necrosis step which was confirmed by DNA fragmentation tests in our experimental study. Indeed, vascular and inflammatory processes contribute to further cell injury and consequently a decline in GFR. Histopathological changes could be due to the accumulation of free radicals as the consequence of the increased H_2O_2 products and lipid peroxidation levels in the kidneys tissue of the DM intoxicated rats.

The present data are in agreement with **Salah *et al.* (2012)** results who showed that histological changes in kidneys revealed by a narrowed Bowman's space, tubular degeneration, tubular cell

desquamation, and tubular dilatation of proximal tubules which accompanied with the biochemical alterations of kidneys.

Several studies have shown that OP insecticides can alter hematologica parameters in experimental animals **(Kalender *et al.*, 2010; Yehia *et al.*, 2007 and Celik *et al.*, 2009)** and in clinical cases **(Patil *et al.*, 2003).** The biochemical alterations of kidneys were also accompained by histological changes in kidneys (**Salah *et al.*, 2012)**.

In general, The results of the present study concluded that the ability of the watermelon rind extracts to inhibit nephrotoxicity induced by dimethoate in rats because watermelon is a rich source of fiber and phytochemical such as phenolics, flavonoids and α - tocopherol, carotenoids such as beta-carotene. These phytochemical have abiological effects like anti-cancer, anti-inflammatory, hepatorenal toxicity, which can be used as therapeutic medicinal agent. Tannins are known to effect the digestive tracts. In addition to the precence of vitamin C which was a water soluble antioxidant essential for strong blood vessels and healthy gums. Its demonstrated cardio-protective action. Combined of vitamin C with other phytochemical was also proven to be protective against dimethoate induced toxicity in the kidneys by preventing oxidative stress and preventing excessive lipid peroxidation by maintaining biochemical indicators at near-normal concentration and improving the kidney's histopathology.

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استخدام قشر البطيخ كعلاج لتخفيف سمية الكلى المستحدثة بمبيد الدايمثوات فى ذكور الجرذان

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أجريت الدراسة الحالية لالقاء الضوء على التأثير الوقائي لقشرة البطيخ ضد تسمم الكلى الناتج عن مبيد الدايمثوات. ولقد تم تقدير التحاليل الأولية لقشر البطيخ المجفف على أساس الوزن الجاف وكذلك بعض التحاليل النباتية الكيميائية في المستخلص المائي والمستخلص الكحولي لقشر البطيخ وأوضحت النتائج أن محتوى الرطوبة والرماد ومحتوى البروتين ومحتوى الدهون ومحتوى الألياف كانت كالآتي 10.61 ، 13.09، 11.17، 2.44 و 17.28% على الترتيب. وكذلك محتوى الفينولات والفلافونيدات والتانينات وفيتامين C وكانت كالآتي 14.88ملجم/جرام، 2.55 ملجم/جرام و2.11 ملجم/100 جرام و 5.65 ملجم/100 جرام على الترتيب في المستخلص المائي وكانت 13.66 ملجم/جرام، 2.34 ملجم/جرام، 1.52 ملجم/100 جرام و 5.22 ملجم/ 100 جرام على الترتيب في المستخلص الكحولي وقد تم تقدير التحاليل الكيميائية الحيوية في سيرم دم الجرذان وأوضحت النتائج زيادة معنوية في مستوى حمض البوليك واليوريا والكيرياتينين في الجرذان التي عوملت بمبيد الدايمثوات بتركيز 1/10 و 1/20 من الجرعة السامة النصفية وذلك بالمقارنة بالمجموعة الضابطة بينما المجموعات المسممة التي عوملت بمستخلصي البطيخ المائي والكحولي وقشر البطيخ المجفف المطحون المضاف للعليقة بنسبة 5% أظهرت نتائجها تحسن كبير وملحوظ في قياس تلك العوامل وكل هذه القياسات توافقت مع الفحوصات الهستولوجية لقطاعات الكلى.

الكلمات الدالة: قشر البطيخ، الدايمثوات، وظائف الكلى