

Study On the effect of moringa leafe on the Rats Kidney Disease**By****Somia abdallah sallam****Sahar osman Elshafai,AbeerNazeah Abed EL Rahman**Department of Nutrition and Food Science. Faculty of Home Economics,
Minufiya. University**ABSTRACT**

This study was conducted to investigate the effect of some moringa leafes on impaired kidney function of injected rats with gentamicin (garamycin amp). Thirteen mature albino rats weighting 150-160g B.wt. each were used, and divided into 6 equal groups, one was kept as a control -ve group, while the other groups were injected by gentamicin 10mg/kg/day for day for day a period of 6 days. The used plants were given as a percent of 3%,5%,7% and9% from the Basel diet kidney functions (urea, creatinin, uric acid), total cholesterol, triglycerides, lipoproteins:(HDL, LDL,VLDL), and histopathological changes of kidney was examined. The obtained results concluding the feeding with the tested plants improved kidney functions and lipid profile. The best percentage of moringa was 7% form the basel diet,Also moringa didn't showany histopatollogical changes in kidney tissue.

Key words;moringa,kidny functions, histopathological changes.

دراسة تأثير أوراق المورينجا علي الفئران المصابه بأمراض الكلي

المستخلص العربي:

تم اجراء الدراسة الحالية لمعرفة تأثيرأوراق المورينجا على الخلل الفيسيولوجي المحدث في الكلي الفئران المصابة بجينتاميسين. تم استخدام ٣٠ فأر أبيض بالغ يتراوح وزن كل منهما من ١٥٠-١٦٠ جم وتم تقسيمهم الى ٦ مجموعات متساوية احدهما كمجموعة ضابطة سالبة أما المجموعات الأخرى فتم احداث الفشل الكلوى فيها بحقن عقار جينتاميسين ٠.٥ بجرعة ممللى من وزن الجسم مره كل يوم ولمدة ٦ ايام. وأضيف النبات المستخدم (مورينجا) بنسبة ٣% و٥% و٧% و٩% لكل منهما من الوجبة الأساسية على هيئة اوراق مورينجا مطحونه. ووظائف الكلي (اليوريا ,الكرياتينين,حمض اليوريك) والكوليستيرول الكلي والجليسيريدات الثلاثية والليبوبروتينات (HDL- LDL- VLDL). وكذلك اجراء فحص الهستوباسولوجى للكلي. وقد أظهرت نتائج هذه الدراسة أن تناول اوراق نبات المورينجا ينتج عنه تحسن فى وظائف الكلي ونسبة الدهون بالدم. وكانت افضل نسبه للمورينجا بنسبة ٧% ولم يحدث تغير في هستوباسولوجى للكلي .

الكلمات المفتاحية: المورينجا- وظائف الكلي- التغيرات الهستوباسولوجية للكلي

Introduction

Kidneys are very important organs . within the human body. They gurad blood volume, filter the blood and form urine ,regulate water ,electrolyte and acid base balance, produce some small hormones and participate in the metabolism of others. At rest ,an estimated 20%of cardiac output (~1000ml/minute)flows through the kidneys where it is filtered and reconditioned.(*Blandy andsedky,1995*) .

In Egypt, the estimated prevalence of end stage renal disease increased from 225 per million population in 1996 (*Afifi,1999*)to 375per million 2001 as report by (*Afifi A,2003*)

Phytotherapy is the treatment and prevention of disease using plants , plants parts, and preparations made from them. The plants traditionally used in phytotherapy are called medicinal plants, or herbs phytotherapy is only one branch of herbal medicine in which this science covers phytochemistry ,phytoarmacy ,phytopharmacology,and phytotherapy (*weiss and fintelmann 2000*)

Medicinal plants have been used by all civilizations as a source of medicines since ancient times. In the recent times, there has been growing interest in exploiting the biological activities of different ayurvedic medicinal herbs, due to their natural origin, cost effectiveness and lesser side effects (*Naik et al., 2003*).

Interest in medicinal plants as a re-emerging health aid in the maintenance of personal health and well-being has been fuelled by rising costs of prescription drugs, and the bioprospecting of new plant-derived drugs (*Sharma et al., 2010*)

Anwer et al.,(2007) family Moringa is an important tropical crop that is used as human food, medicine and in oil production Among myriad of plants, Moringa oleifera Lam is one of the best known and most distributed species of Moringaceae

Moringa's effectiveness is known for treating more than 300 conditions and has been heavily utilized in folk medicine to treat a variety of health conditions. It has been targeted on the Discovery Channel as one of the best all natural supplements in the world (*Shukla et al; 1998*).

Moringa Oleifera lam (also known as Malunggay) is a highly valued plant, distributed in many countries of the tropics and subtropics Different parts of this plant contain a profile of important minerals, and are a good source of protein, vitamins, beta-carotene, amino acids and various phenolics. The Moringa plant provides a rich and rare combination of zeatin, quercetin, beta-sitosterol, caffeylquinic acid and kaempferol. In addition to its compelling water purifying powers and high nutritional

value, *Moringa Oleifera* is very important for its medicinal value (**Shukla et al; 1998**).

Adesokan et al;(2007) Various parts of mor such as the leaves, roots, seed, bark, fruit, flowers and immature pods act as cardiac and circulatory stimulants, possess antitumor, antipyretic, antiepileptic, anti-inflammatory, antiulcer, antispasmodic, diuretic, antihypertensive, cholesterol lowering, antioxidant, antidiabetic, hepatoprotective, antibacterial and antifungal activities, and are being employed for the treatment of different ailments in the indigenous system of medicine, particularly in South Asia.

More so *Moringa* trees have been used to combat malnutrition, especially among infants and nursing mothers. A large number of reports on the nutritional qualities of *Moringa* now exist in both the scientific and the popular literature. Leaves can be eaten fresh, cooked, or stored as dried powder for many months without refrigeration, and reportedly without loss of nutritional value (**Trees for life, 2005**).

Health nutritionists claim that an ounce of malunggay has the same vitamin c content as seven oranges. In fact, it has been recognized and accepted by the US Food and Drug Administration as one of the four dietary antioxidants, the others being vitamin E, beta-carotenes and selenium. (A dietary oxidant is a substance in food that significantly decreases the adverse effects of harmful chemicals). Countless instances of life saving nutritional rescue that are attributed to *Moringa* (**Udupa, et al; 1994**)

There have been claims that *Moringa* can be used to lower blood pressure, aid the pains caused by rheumatism, headaches and migraines, as well as its being an antitumor plant. Malunggay is also used for purgative and antifungal purposes, as well. All these prove the claim that this plant is indeed multipurpose (**Trees for life, 2005**).

MATERIALS:

The used plants:

Moringa leaves obtained from the Society of Friends in Sadat City.

preparation of rats with impaired kidney:

Impaired kidney can be induced in normal healthy male albino rats by intra-peritoneal injection of gentamicin(aminoglycosides antibiotics) obtained by Memphis Co. form Pharm .Chem.Ind.Cairo.A.R.E.at 10 mg/kg/day for 6days in which the nephrotoxicity,one of the adverse reaction of gentamicin takes place.

Rats:

Thirteen adult male albino rats, weighting 150-160g. from Medical Insects Research Institute, Doki, Cairo, were used in this study.

Rats were housed in wire cages under the normal laboratory condition and were fed on standard diet for a week as an adaptation period. Diet was offered to rats in special food cups to avoid loossor conditions of food, water was provided to the rats by glass tubes supported to one side of the cage, food and water provided ad-labium and checked daily.

EXPERIMENTAL DESIGN:

The experimental was done in the Faculty of Home Economic, Minufiya University, Shebin El-kom. Rats were housed in wire cages in a room temperature about 25 C° and kept under normal healthy conditions.

RATS WERE DIVIDED INTO THE FOLLOWING GROUPS:

GROUP 1: NEGATIVE CONTROL GROUP- NORMAL GROUP- (6 RATS).

In this group rats were kept on standard diet and tap water.

GROUP 2: HYPERCHOLESTEROLEMIC GROUP)52 RATS(.

In this group, rats were induced kidney failure by intra-peritoneal injection of gentamicin(aminoglycosides antibiotics) to induce kidney failure. This group was subdivided into 5 subgroups to fed on the experimental diets for (4) weeks according to the following:

Group(a): 5rats: positive control group (untreated group)

Group (b): 5rats: treated with 3% moringa.

Group (c): 5rats: treated with 5% moringa.

Group (d): 5rats: treated with 7% moringa

Group (e): 5rats: treated with 9% moringa.

BLOOD SAMPLING AND ORGANS:

Blood samples were collected after 12 hours fasting at the end of the experiment using the abdominal aorta in which the rats were scarified under ether anetheized. Blood samples were received in to clean dry centerfuge tubes and left to clot at room temperature, then centerfused for 10 minutes at 3000 rpm to separate the serum. Serum was carefully aspirate, transferred in to clean cuvet tubes, and stored frozen at -20°c for analysis. All serum samples were analyzed for determination the following parameters:

urea, creatinin, uric

acid, Lipid profile Cholesterol, triglycerides (T.G), (LDL), (HDL), (VLDL), At the same time, the organs: kidney was removed, washed in salin solution, dried by filter paper, weighted, and stored frozen in formalin solution 10% for histopathological testing according to method mentioned by *Drury and Wallington, (1980)*.

Results and Discussion

Table (1) and fig (1) show fasting serum urea, creatinin, and uric acid (mg/dl) for negative control, positive control, and kidney failure treated groups with (M3, M5, M7 and M9). As shown in this table, *urea* of negative control were high ($p < 0.05$) comparing with positive control which were (23.125 ± 11.433) and (18.15 ± 2.537) respectively, the mean value of all kidney failure treated groups were less compared with positive control, but these results did not show any significant differences.

This results agree with (H.C.C Maduka et al., 2014) From the result, there was significant increase in the level of urea in the blood in acetaminophen-induced control Wister albino rats (group B) compared with the normal control group (A) and the other extract treated groups (C, D, E and F) suggesting that the extract reduced the metabolism and eventual excretion of urea.

This result disagree with (Halaby and Elmetwaly et al., 2013) showed that the level of urea decreased gradually according to the concentration of moringa

This result disagree with with (Lakshmane et al., 2013) showed that moringa pterygosperma treatment reduced the elevated urea level in experimental rats.

As for *Creatinin*, the mean value of (*Creatinin*) for negative control was low significant ($p < 0.05$) comparing with positive control which was (1.11 ± 0.345) and (1.075 ± 0.298) respectively. Whereas the mean value of kidney failure treated groups with (M3, M5, M7 and M9%) were (0.875 ± 0.298) , (0.692 ± 0.086) , (0.675 ± 0.095) and (0.875 ± 0.095) , respectively and were lower when compared with positive control.

These results agree with (Halaby and Elmetwaly et al., 2013) showed that the level of creatinine decreased gradually according to the concentration of moringa.

These results agree with (Lakshmane et al., 2013) showed that moringa pterygosperma treatment normalizes the elevated creatinine level in experimental rats..

These result agree with (H.C.C. Maduka et al., 2014) found, the extract reduced the level of creatinine in extract treated groups (groups C, D, E and F) compared with the acetaminophen-treated control group (group B) at $P < 0.05$ suggesting cytoprotection effect.

On the other hand the mean value of *uric acid*, for negative control was higher significant ($p < 0.05$) comparing with positive control which was (4.25 ± 0.714) (2.47 ± 0.434) respectively. Also, the mean value of group of kidney failure treated groups with (M3 and M9) were (2.7 ± 0.761) and (2.325 ± 0.325) , respectively and were lower ($p < 0.05$) as compared with

positive control, whereas the mean value of kidney failure treated groups with (M5 and M7) were (3.675 ± 1.839 and 3.25 ± 0.818), respectively was higher ($p < 0.05$) as compared with positive control.

These results agree with (Halaby and Elmetwally *et al* 2013) there was significant difference between positive control group and group fed on Moringa fortified bread at 15%, which reduced the uric acid level significantly. The concentration of uric acid was reduced by 2.84% and 18.41% respectively they reported that there in groups M9 and M3 but groups which feed on M5 and M7 disagree with.

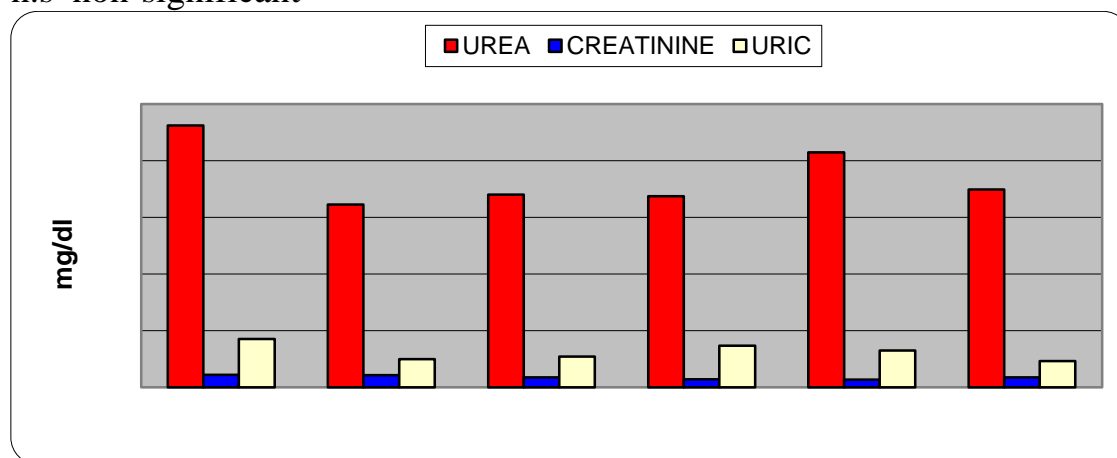
Table(1): fasting serum urea, creatinin, and uric acid for negative control, positive control, and kidney failure treated groups with (m3, m5, m7, m9)

Varbles	C-	C+	M3	M5	M7	M9	Sig	LSD
	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd		
UREA	23.125 ^{a±} 11.537	16.15 ^{a±} 2.357	17.025 ^{a±} 0.865	16.875 ^{a±} 2.393	20.75 ^{a±} 2.986	17.475 ^{a±} 2.041	n.s	7.8772
CREATININE	11.1 ^{a±} 0.345	1.075 ^{a±} 0.298	0.875 ^{ab±} 0.15	0.692 ^{b±} 0.086	0.675 ^{b±} 0.095	0.875 ^{ab±} 0.095	*	0.3001
URIC ACID	4.25 ^{a±} 0.714	2.475 ^{b±} 0.434	2.7 ^{ab±} 0.761	3.675 ^{ab±} 1.839	3.25 ^{ab±} 0.818	2.325 ^{b±} 0.320	*	1.5329

Means in the same row with different letters are significantly differences.

*high significant ($p < 0.05$)

n.s non-significant



FIG(1): fasting serum urea, creatinin, and uric acid for negative control, positive control, and kidney failure treated groups with (m3, m5, m7, m9)

Table (2) and fig (2a, 2b) show fasting serum lipids for negative control, positive control, and kidney failure treated groups with (M3, M5, M7 K and M9). As shown in this table, the mean value of (T.G) the mean value of negative control was higher than positive control by mean (145.47 ± 20.432) and (143.95 ± 16.413) (mg/dl), respectively. whereas the mean value of kidney failure treated groups with (M3, M7 and M9) were (127.9758 ± 8.619 , 120.55 ± 6.532 and 115.9 ± 6.112), respectively. significantly lower ($p < 0.05$) when compared with positive control, while the mean value of

kidney failure treated groups ,and M5(5%)wrer(173 ± 58.571),respectively. show any significant higher as compared with positive control.

This result agree with **Jain et al., (2010)** found that the serum triacylglyceride, atherogenic index were reduced by M. Oleifera

This result disagree with **Okwari et al.,(2013)** The TG concentration showed a significant increase ($P < 0.05$) in all the test groups when compared with the control.

As for (**T.c**) the mean value of negative control was high than positive control by mean (73.135 ± 14.18) and (65 ± 1.825) (mg/dl), respectively. whereas the mean value of kidney failure treated groups with (M3,M5,M7 and M9) were (66.875 ± 5.573 , 73.625 ± 2.973 , 68.925 ± 6.833 and 73.025 ± 4.791),respectively. significantly higher ($p < 0.05$) when compared with positive control.

This result disagree with (**Reddy et al.,2012**) they showed reduction in cholesterol levels in rats on oral supplementation of MO leaves powder.

This result disagree with (**Halaby and ELmetwaly et al.,2013**) MO was also found to increase the excretion of fecal cholesterol. Thus, it can be concluded that MO possesses a hypolipidemic effect.

This result disagree with (**Okwari et al.,2013**) The TC increased significantly ($P < 0.05$) in all the test groups with the exception of the TPO which showed a significant ($P < 0.05$) decrease compared with the control

As, for (**LDL**), the mean value of negative control was lower than positive control by mean (67.825 ± 1.512) and (86.05 ± 17.383) (mg/dl), respectively. Whereas the mean value of kidney failure treated groups with (M3, M5 and M7)were (82 ± 9.546 , 69.125 ± 9.245 and 76.5 ± 12.822).respectively. Significantly lower ($p < 0.05$) when compared with positive control, while the mean value of kidney failure treated groups with(M9)were(91.2 ± 17.79), respectively. Show any significant higher as compared with positive control.

This result agree with **Jain et al., (2010)** found that the serumLDL, atherogenic index were reduced by M. Oleifera

This result disagree with (**Okwari et al.,2013**) The results of low density lipoprotein-cholesterol (LDL-C) showed a significant ($P < 0.05$) reduction in LDL-C in all the test groups except TPO which showed a significant ($P < 0.05$) increase when compared with the control .

As, for **HDL** the mean value of negative control was lower than positive control by mean(75.4 ± 11.745)and(75.35 ± 10.511) (mg/dl), respectively. While the mean value of kidney failure treatedgroups with(M3,M5,M7andM9)were(80.4 ± 9.969 , 80.6 ± 10.871 , 78.925 ± 4.382 and 83.75 ± 14.097), respectively. significantly high($p < 0.05$) when compared with positive control.

This result disagree with (Okwari et al.,2013) The HDL-C reveals a significant ($P<0.05$) elevation in all the experimental groups with the exception of TPO which showed a significant ($P<0.05$) decrease when compared with the control

This result agree with Jain et al., (2010) HDL level was increased as compared to the corresponding high fed cholesterol diet group (control).

On the other hand the mean value of *VLDL*, for negative control was less significant ($p<0.05$) comparing with positive control which was (0.385 ± 0.042) (0.432 ± 0.221) respectively. Also, the mean value of group of kidney failure treated groups with (M3,M5,M7andM9)were $(0.39\pm 0.011, 0.425\pm 0.031, 0.415\pm 0.020$ and 0.425 ± 0.028), respectively and was lower ($p<0.05$) as compared with positive control.

This result disagree with (Okwari et al.,2013) The results of very low density lipoprotein-cholesterol (VLDL) showed a significant ($P<0.05$) increase in all the test groups when compared with the control

Table(2):fasting serum lipids for negative control ,positive control, and kidney failure treated groups with(m3,m5,m7,m9)

Varbles	C-	C+	M3	M5	M7	M9	Sig	Lsd
	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd	mean±sd		
Triglycrids	145.475 ^a ± 20.432	143.95 ^{ab} ± 16.413	127.975 ^{ab} 8.619	173 ^a ± 58.571	120.55 ^b ± 6.532	115.9 ^b ± 6.112	*	42.0413
Cholesterol	73.135 ^a ± 14.18	65 ^a ± 1.825	66.875 ^a ± 5.573	73.625 ^a ± 2.973	68.925 ^a ± 6.833	73.025 ^a ± 4.791	n.s	11.5441
LDL	67.825 ^b ± 1.512	86.05 ^{ab} ± 17.383	82 ^{ab} ± 9.546	69.125 ^b ± 9.245	76.5 ^{ab} ± 12.822	91.2 ^a ± 17.79	*	19.361
HDL	75.4 ^a ± 11.745	75.35 ^a ± 10.511	80.4 ^a ± 9.979	80.6 ^a ± 10.871	78.925 ^a ± 4.382	83.75 ^a ± 14.097	n.s	15.5636
VLDL	0.385 ^a ± 0.042	0.432 ^a ± 0.221	0.39 ^a ± 0.011	0.425 ^a ± 0.031	0.415 ^a ± 0.020	0.425 ^a ± 0.028	n.s	0.04378

Means in the same row with different letters are significantly differences.

*high significant($p<0.05$)

n.s non-significant

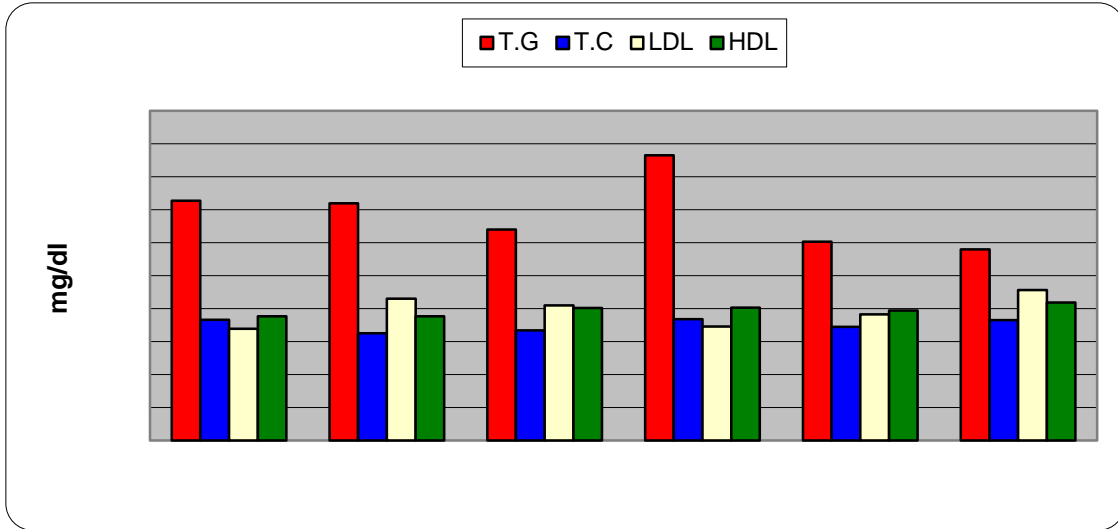


fig (2a):fasting serum T.G,T.C,LDL and HDL for negative control ,positive control,and kidney failure treated groups with(m3,m5,m7,m9)

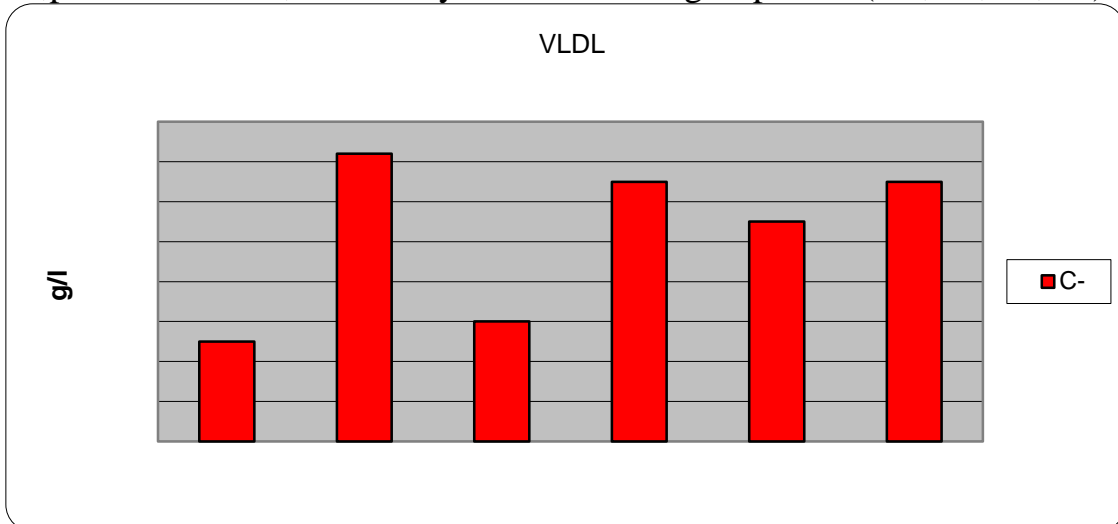


fig (2b):fasting serum VLDL for negative control ,positive control,and kidney failure treated groups with(m3,m5,m7,m9)

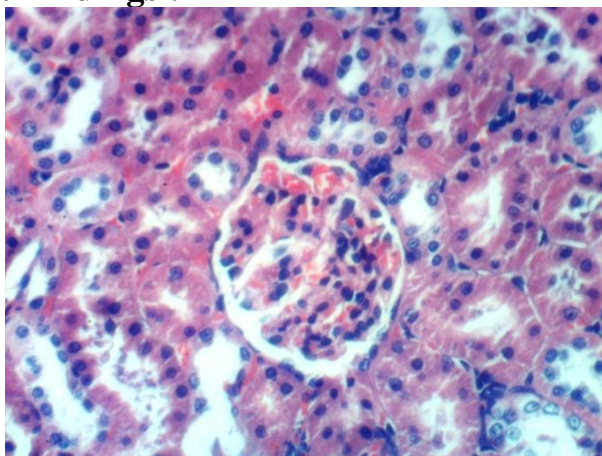
Histopathological findings :

Fig. (1): Kidney of rat from group 1 showing the normal histological structure of renal parenchyma (H & E X 400).

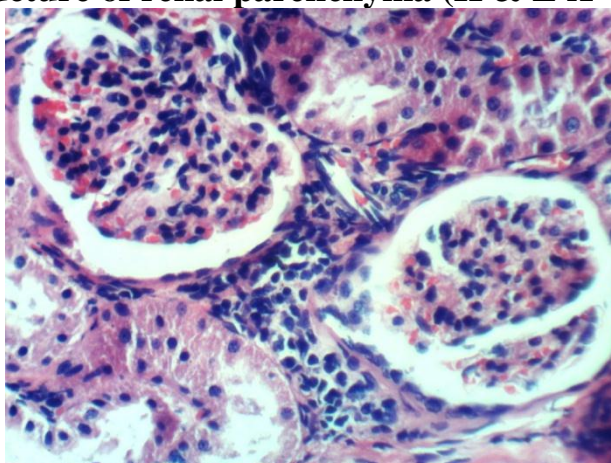


Fig. (2): Kidney of rat from group 2 showing focal necrosis of renal tubules associated with inflammatory cells infiltration (H & E X 400).

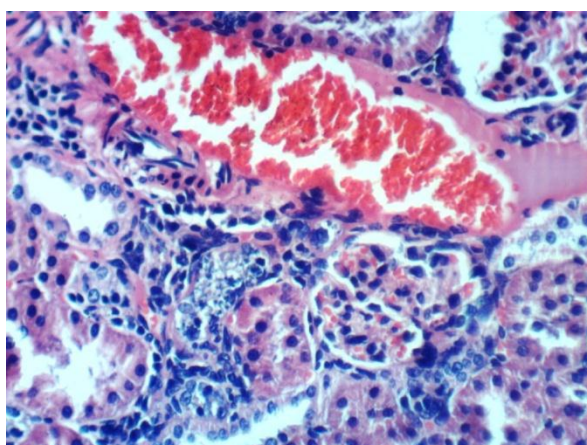


Fig. (3): Kidney of rat from group 2 showing interstitial nephritis (H & E X 400).

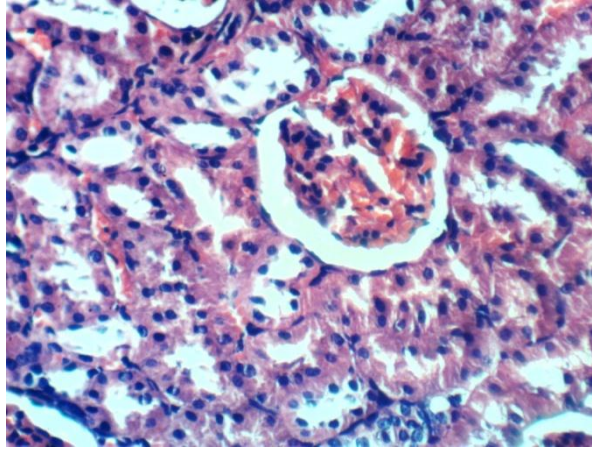


Fig. (4): Kidney of rat from group 3 showing no histopathological changes (H & E X 400).

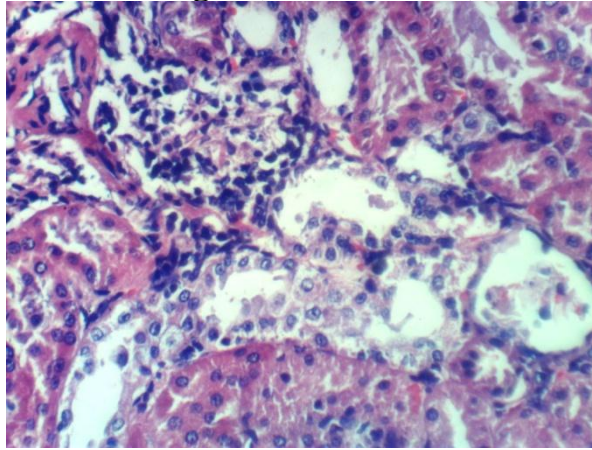


Fig. (5): Kidney of rat from group 4 showing focal necrosis of renal tubules associated with inflammatory cells infiltration (H & E X 400).

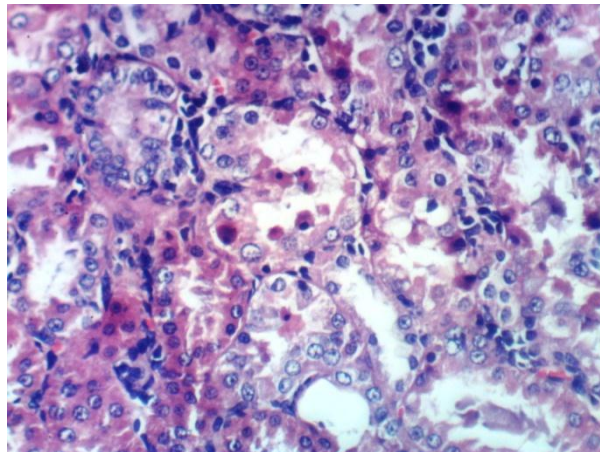


Fig. (6): Kidney of rat from group 4 showing interstitial nephritis and presence of epithelial cast in the lumen of renal tubules (H & E X 400).

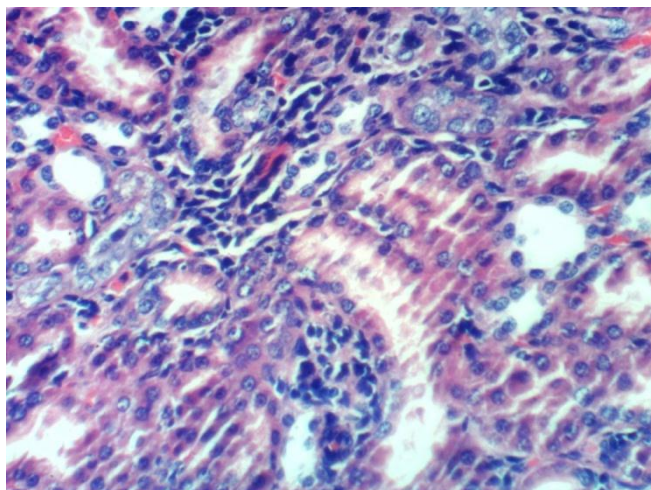


Fig. (7): Kidney of rat from group 5 showing interstitial nephritis (H & E X 400).

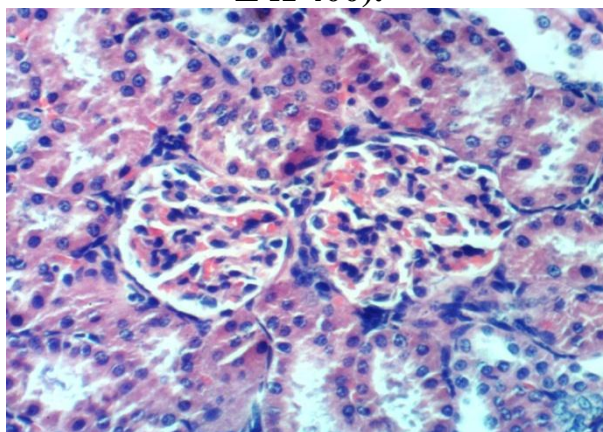


Fig. (8): Kidney of rat from group 5 showing no histopathological changes (H & E X 400).

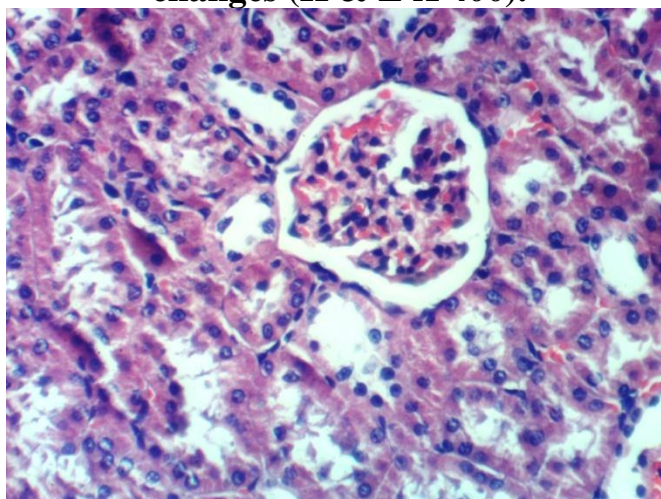


Fig. (9): Kidney of rat from group 6 showing no histopathological changes (H & E X 400).

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