The Egyptian Journal of Anatomy, June 2020; 43(2): 387-411

Original Article	**Broccoli extract protects against induced renal cortical damage by hemolytic anemia in adult male rats**

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ABSTRACT

Introduction: Hemolytic anemia can cause systematic body dysfunctions. Hemoglobin clearance occurred by kidneys after saturation of natural scavenging systems. Broccoli extract is effective in treatment of human disorders as an antioxidant.

Aim: To assess the protective effect of Broccoli extract on renal cortical damage induced by hemolytic

Material and Methods: Forty eight adult male rats were divided into five groups. Group I (control group): eighteen rats subdivided into three equal subgroups. Group II: twelve rats received Phenylhydrazine in a dose 60 mg/kg via intraperitoneal injection then divided into two equal subgroups. Group III: six rats received Broccoli extract 200 mg/kg via an oral gavage. Group IV: six rats' received Phenylhydrazine then Broccoli at a dose and route similar to the previous groups. Group V: six rats received Broccoli then Phenylhydrazine at a dose and route similar to the previous groups. At the end of the experiment, blood samples were collected then kidneys were sampled for histopathological and ultrastructural studies.

Results: Examination of renal cortex sections from group IIa showed shrunken glomerulus, thickened parietal layer of Bowman's capsule, shedding of the tubular epithelium, vacuolation, cellular infiltration and congested capillaries. Electron microscopic examination revealed disorganization of renal cortex. On the other hand, examination of group IV and V section showed apparent improvement in almost all layers.

Conclusion: Broccoli extract had a protective effect better than the curative role against induced renal cortex damage by hemolytic anemia.

Received: 10 January 2020, **Accepted:** 16 January 2020

Key Words: Broccoli extract, hemolytic anemia, phenylhydrazine, prophylactic treatment, renal cortex damage.

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The Egyptian Journal of Anatomy, ISSN: 0013-2446, Vol. 43 No. 2

INTRODUCTION

Anemia is a serious global public health problem associated with an increased risk of morbidity and mortality. It affects over 30% of the world's population especially in developing countries[1, 2].

Hemoglobin (Hb) is a hemoproteins which responsible for body's homeostasis[3]. Each globin contains a haem group and iron atom inside[4].

In certain pathological conditions such as sickle-cell disease, autoimmune hemolytic anemia, mechanical heart valve-induced anemia, these molecules are released into the blood stream and accompanied by the release of pro-oxidative and pro-inflammatory components[4, 5].

Hemolytic anemia and resultant hypoxia can cause systematic body dysfunctions[6]. It is a common hallmark of kidney disease which is associated with reduced quality of life and mortality. It has also been shown that anemia is an independent predictor for ischemic cardiac events[7].

The most frequently used method to induce hemolytic anemia in experimental animals is the injection of Phenylhydrazine (PHZ). It induces hemolytic anemia and intravascular hemolysis by the interaction between PHZ and hemoglobin

DOI: 10.21608/EJANA.2020.22235.1065

to form hydrogen peroxide with formation of oxidized derivatives and free radicals of hydrazine[5].

Moreover, PHZ has been reported to induce Heinz body formation, oxidative degradation in the erythrocyte membrane and destabilizing the globin portion leading to release of globin-free heme[8,9].

These characteristics of PHZ have drawn the attention of the researchers to consider it as an effective drug for studying the hemolytic anemia in experimental models[9].

On the other hand, Brassica oleraceae varitalica (Broccoli) belongs to family Brassicacea, known as "Crown Jewel of Nutrition"[10]. It is rich in potential health boosting components like vitamins (A, C, folic acid), minerals (Na, K, Ca, phosphorus), dietary fibres, low in saturated fat, and very low in cholesterol[11, 12].

The edible parts of broccoli are sprouts commonly called as inflorescence Brassica oleraceaevar italic[10]. It has high water content (89.30%) and is low in fat (0.37%). Inflorescence was reported to have anticancer, antioxidant, antiseptic, antiulcer, hypoglycemic activities[11, 12].

Brassica oleracea was considered to be effective in treatment of variety of human disorders caused by oxidative stress[10, 13].

The present study was conducted to evaluate the possible protective and antianemic properties of aqueous extract of Brassica oleraceae var italica inflorescences (Broccoli extract) on the kidney as either curative or prophylactic treatment in case of PHZ induced hemolytic anemia in rats and the possible spontaneous regeneration capacity of the kidney if left without treatment for 14 days.

MATERIAL AND METHODS

Experimental animals

Forty eight adult male albino rats of Wister strain weighing 180-200gram were used in the present study. Animals were obtained from the animal house of the Medical Research Center, Faculty of Medicine, Ain Shams University.

Rats were allowed free access to water and food and were housed in suitable cage with 12 hours light/dark cycle, good hygienic conditions, good ventilation. Animals were left one week for acclimatization before the start of the experiment.

All the experiments were conducted in accordance with the national guidelines approved by the Committee of Animal Research Ethics, Ain Shams University, Faculty of Medicine.

Chemicals:

• Phenylhydrazine: liquid (PHZ; Sigma Chemical Co., St. Louis, MO, USA).

• Broccoli extract: Tablet containing powder (500mg) from NATURE'S ANSWER. /USA.

Experimental design

At the start of the study, blood samples were collected from all rats through puncture of the retro-orbital plexus using capillary tube to exclude anemia or kidney diseases.

Rats were divided randomly into five groups as follow:

Group I (control group): eighteen rats subdivided into three equal subgroups:

Ia: six rats were not subjected to any procedure and served as a control.

Ib: six rats were received for two days intraperitoneal injection of 1 ml normal saline, diluting vehicle for PHZ.

Ic: six rats were received 1 ml normal saline by oral gavage for 14 days, diluting vehicle for Broccoli extract.

Group II (PHZ group): included twelve rats and used to induce experimental hemolytic anemia.

Each rat was received PHZ in a dose 60 mg/kg body weight /day dissolved in sterilized normal saline by intraperitoneal injection given for two days. This dose was selected based on previous studies[14].

Solution of PHZ was prepared immediately before use[10].

Then rats subdivided into two subgroups (six rats in each one):

• IIa (PHZ treated group): On day 4 after injection blood samples were collected then rats were sacrificed.

• IIb (spontaneous recovery group): rats were sacrificed at the end of the experiment.

Group III (Broccoli group): six rats received Broccoli extract at dose of 200 mg/kg body weight/day dissolved in normal saline by oral gavage for 14th days. This dose was selected based on previous studies[10].

Group IV (PHZ-broccoli group): six rats received PHZ for two days then received Broccoli extract for 14th days at a dose and route similar to the previous groups.

Group V (Broccoli-PHZ group): six rats received Broccoli extract for 14th days then received PHZ at a dose and route similar to the previous groups.

• Rats mean body weight were measured twice weekly from the beginning to the end of the study to adjust PHZ and Broccoli dose.

• At the end of experiment, all rats fasted overnight, then anesthetized by ether inhalation. Blood samples were collected from puncture of the retro-orbital plexus using capillary tubes. Under the anesthesia, the abdominal wall was retracted and both kidneys were removed, washed with cold saline.

Biochemical studies:

At the onset of the study, a blood sample was collected from puncture of the retro-orbital plexus using capillary tube for the measurement of hematological and kidney parameters levels to exclude anemia or kidney diseases.

At the end of experiment, blood samples were collected from all groups except group IIa which sampled at day 4. Blood samples collected for measurement of hematological parameters (hemoglobin (Hb), packed cell volume (PCV), red blood cells (RBC's), white blood cells (WBC's), mean corpuscular volume (MCV) and mean corpuscular hemoglobin (MCH), urea and creatinine serum level.

All blood samples were processed at Tumor Markers Oncoloy Research center, Al-Azhar University.

Light microscopic study:

Kidneys were dissected longitudinally into two halves and placed in 10 % formalin. The samples were dehydrated in ethanol and fixed to form paraffin blocks. The blocks were cut into 5-µm thick sections.

Sections were stained with the following:

• Hematoxylin–Eosin (Hx & E)

• Periodic acid Schiff (PAS): for demonstration of parietal layer of Bowman's capsule, basement membrane of proximal and distal convoluted tubules and brush border of proximal convoluted tubules[15].

• Immunohistochemical study (Heme oxygenase 1 (HO-1)): 3-µm-thick kidney sections were cut with Cryostat Leica AS-LMD. HO-1 expression was studied using rabbit anti-mouse HO-1 followed by a polymer anti-rabbit IgG-HRP. Staining was revealed with DAB solution[5].

All sections were examined with the light microscope and photographed with Lecia ICC50 W camera.

Ultrastructural study:

Kidney samples were obtained and cut into 1 mm3. Preparing and sectioning of ultra-thin sections were carried out[16]. Sections were stained by uranyl acetate and lead citrate.

Ultra –thin sections were examined under a TEM (JEOL 1200 EX II, Japan) at the Regional Mycology and Biotechnology Centre, Al-Azhar University, Egypt.

Statistical Analysis:

The hematological and kidney data obtained (Hb, PCV, RBC's, WBC's, MCV, MCH, serum

creatinine level and urea) were recorded and subjected to statistical analyzed.

Data were expressed as mean and standard deviation for the quantitative variable. Data were statistically analyzed using statistical package Statistical Package for Social Sciences version 16 (SPSS Inc., Chicago, USA).

Comparisons between groups were done using ANOVA (analysis of variance) followed by Post Hoc test for multiple comparisons between each 2 groups. The results were considered statistically significant when *p value < 0.05* and highly significant when *p value <0.001*.

RESULTS

There were no signs of morbidity or mortality recorded in the experimental animals. Moreover, there were no statistically significant differences between biochemical, histological, immunohistochemical and ultrastructural results between control subgroups (group Ia, Ib and Ic) or between control group and Broccoli group (group I and group III) so all will be mentioned in results as group I.

• *Biochemical studies:*

The mean values ±SD and comparative charts were mentioned in tables and histograms respectively.

a) Hb:

There was a highly statistically significant decrease in group IIa Hb in comparison to group I and non-statistically significant difference between group IV and V to group I.

In comparison to group IIa, there were statistically significant increases in group IIb Hb and group V Hb with highly statistically significant increase in group IV Hb (Table 1, Histogram 1).

b) PCV:

In comparison to group I, group IIa showed statistically significant decrease while group IV showed statistically significant increase and non-statistically significant difference between group V and group I.

Group IIb PCV and group IV PCV were non-statistically significant increase when compared to the group IIa. Group V data was statistically significant increase when compared to the group IIa (Table 1, Histogram 2).

c) RBC's:

There was highly statistically significant decrease in group IIa when compared to the group I accompanied with statistically significant decrease in group IV RBC's and non-statistically significant decrease in group V RBC's.

In comparison to group IIa, group IIb and group IV showed statistically significant increase while group V showed a highly statistically significant increase (Table 1, Histogram 3).

d) WBC's:

In comparison to group I, there was statistically significant increase in group IIa WBC's, a highly statistically significant increase in group IV and non-statistically significant increase group V.

While in comparison to group IIa, there were statistically significant decrease in group IIb, group IV and group V (Table 1, Histogram 4).

e) MCV:

Group IIa showed a highly statistical significant increase when compared to the group I while group IV, V showed non-statistically significant difference.

In comparison to group IIa, group IIb showed non-statistically significant difference, group IV showed a highly statistically significant increase while group V showed statistical significant decrease (Table 1, Histogram 5).

f) MCH:

In comparison to group I, group IIa showed a highly statistically significant increase while group IV and group V showed non-statistically significant difference.

Group IIb and group IV showed non-statistically significant difference when compared to the group IIa but group V showed statistical significant decrease when compared to the group IIa (Table 1, Histogram 6).

g) Urea:

There were non-statistically significant difference in group IIa and group V when compared to the group I but group IV showed a statistically significant difference to group I.

In comparison to group IIa, group IIb showed statistically significant decrease, group IV a highly statistically significant decrease and group V showed non-significant statistical decrease (Table 2, Histogram 7).

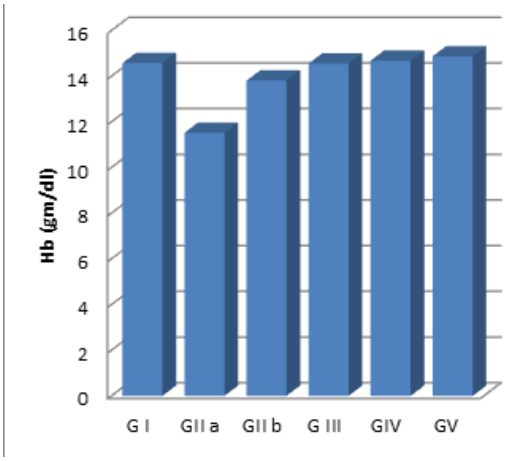
h) Serum Creatinine:

In comparison to group I, group IIa showed a highly statistically significant increase, group IV a statistically significant increase and group V showed non-significant statistical increase.

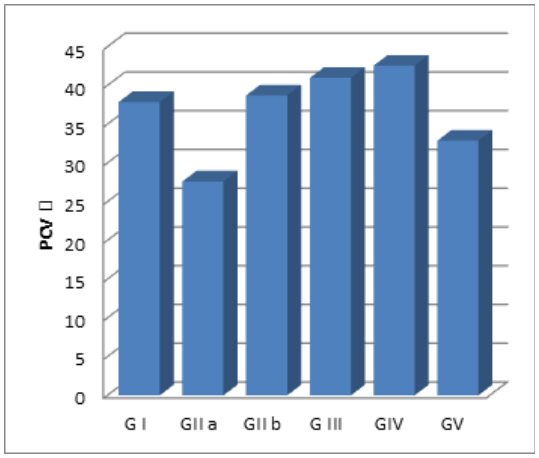
In group IIb, S. Creatinine showed non-statistically significant decrease when compared to the group IIa. While group IV and group V showed a statistically significant decrease when compared to the group IIa (Table 2, Histogram 8).

Table 1a: Effect of Broccoli extracts on hematological parameters in PHZ-induced hemolytic anemia in rat

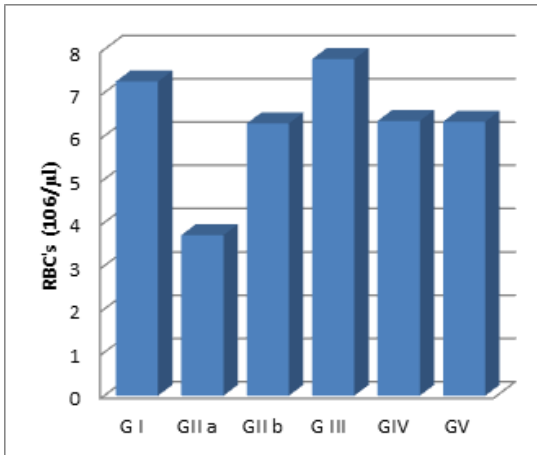
	Hb (gm/dl)	PCV %	RBC (106/µl)	WBC(103/µl)
G I	14.57± 0.45	37.85±5.15	7.26±0.88	4.02±1.02
G IIa	11.52±0.35**	27.61±0.24*	3.71±0.22**	27.81±4.02*
G IIb	13.8±0.84a	38.72±0.21	6.29±0.45a	7.92±0.39a
G III	14.55±0.4	40.97±0.03	7.78±0.32	4.33±0.47
G IV	14.68±0.84b	42.55±1.76*	6.35±1.22*,a	6.15±1.69**,a
G V	14.87±0.33a	32.87±0.97a	6.33±0.48b	22.95±0.98a



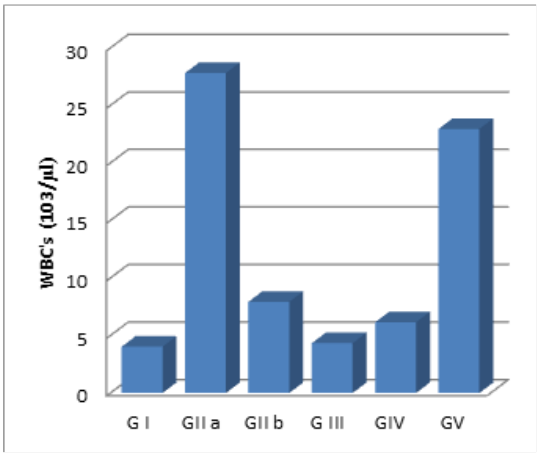
Histogram 1: Effect of Broccoli extract on hemoglobin (Hb) concentration in PHZ induced anemic rats.



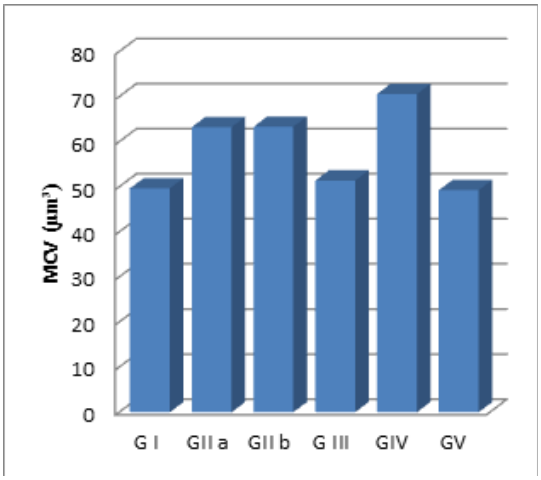
Histogram 2: Effect of Broccoli extract on packed cell volume (PCV) in PHZ induced anemic rats.



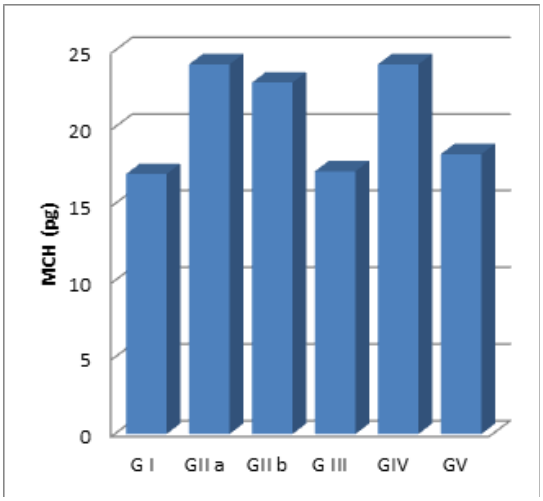
Histogram 3: Effect of Broccoli extract on red blood cell count (RBC's) in PHZ induced anemic



Histogram 4: Effect of Broccoli extract on white blood cell count (WBC's) in PHZ induced anemic rats.



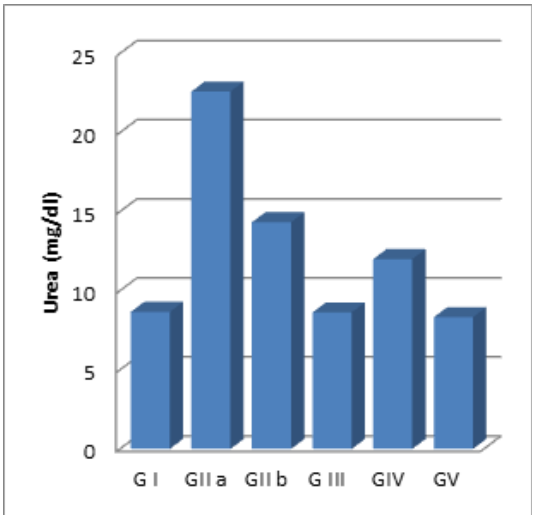
Histogram 5: Effect of Broccoli extract on mean corpuscular volume (MCV) in PHZ induced anemic rats.



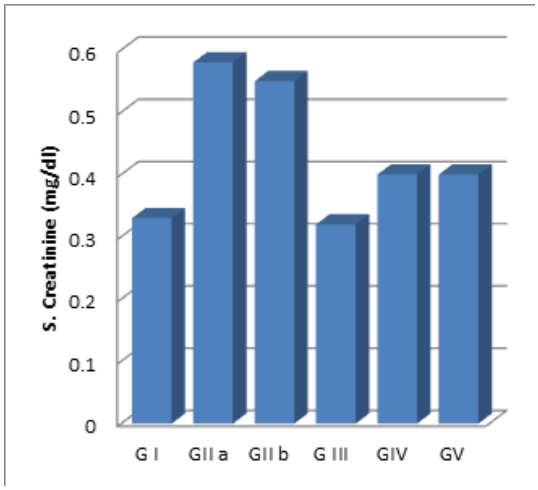
Histogram 6: Effect of Broccoli extract on Mean corpuscular hemoglobin (MCH)in PHZ induced anemic rats.

Table 1b: Effect of Broccoli extracts on hematological parameters in PHZ-induced hemolytic anemia in rat

	MCV (µm3)	MCH (pg)
GI	49.67± 2.74	16.95±0.67
GIIa	63.27±2.55**	24.1±1.64**
GIIb	63.33±1.32	22.92±1.09
GIII	51.4±0.2	17.13±1.6
GIV	70.6±0.14[b]	24.1±0.14
GV	49.27±0.14[a]	18.25±1.63[a]



Histogram 7: Effect of Broccoli extract on urea in PHZ induced anemic rats.



Histogram 8: Effect of Broccoli extract on serum creatinine in PHZ induced anemic rats.

• *Histological results*

Group I (control group):

Light microscopic examination of hematoxylin and eosin (Hx&E) kidney stained sections of group I showed that the renal cortex contained proximal convoluted tubules, distal convoluted tubules and renal corpuscles.

Each renal corpuscle was composed of a tuft of capillaries; the glomerulus surrounded by a double-walled epithelial Bowman's capsule and in-between is the capsular space. The proximal convoluted tubules (PCTs) have narrow lumen and were lined with cuboidal epithelial cells with acidophilic cytoplasm and oval nuclei. The distal convoluted tubules (DCTs) had wider lumen and were lined with cuboidal epithelial cells with apical situated nuclei and acidophilic cytoplasm (Fig. 1A). Periodic acid Schiff stained section showed PAS positive reaction in the brush borders of the cells lining the PCTs, as well as in the Parietal layer of Bowman's capsule and the basement membranes of the renal tubules (PSTs, DCTs) (Fig. 1B). Immunohistochemical study showed negative cytoplasmic HO−1 staining in the tubules and glomeruli (Fig. 1C).

Transmission electron microscopic examination of renal ultrathin sections of group I showed PCTs with apical brush border containing euchromatic nucleus with prominent nucleolus and numerous mitochondria (Fig. 2A). The DCTs had large vesicular nucleus, short scattered microvilli, mitochondria and endoplasmic reticulum (Fig. 2B). The glomerulus showed capillaries with electro lucent basement membrane containing red blood cells. The foot processes of the podocytes were intact in the glomeruli. Mesangial cell with a large vesicular nucleus was also noticed (Fig. 2C).

Group IIa (PHZ group):

Examination of H&E stained sections obtained from PHZ treated group for a period of four days revealed loss of tubular architecture in many areas. Glomerulus was shrunken with widening of the capsular space and thickened parietal layer of Bowman's capsule. PCTs showed detachment of the epithelial lining of some tubules. DCTs showed homogenous acidophilic hyaline casts in the lumen. Cytoplasmic vacuolation and shedding of the tubular epithelial cells inside the lumen were seen. Interstitial mononuclear cellular infiltrate and dilated, congested peritubular capillaries were noticed accompanied with extravasation of RBCs in the interstitium (Fig. 3A). Periodic Acid Schiff stained sections showed partial loss of the brush border of the proximal tubular cells with loss of PAS reaction of many tubular basement membranes. Thickening of parietal layer of Bowman′s capsule was also noticed (Fig. 3B). Immunohistochemical study showed increase in cytoplasmic HO−1 staining in tubules and glomeruli (Fig. 3C).

Electron microscopic examination of sections of group IIa revealed that the PCTs showed compressed basement membrane that appeared as a single electron dense layer, the cytoplasm

contained shrunken nucleus and bizarre shaped mitochondria. Brush border was less compact compared to that in the control. Some vacuoles were seen and Hem protein deposition was noticed as electron dense patches. (Fig. 4A). The DCTs showed shrunken nucleus and peripheral condensed chromatin, multiple mitochondria and microvilli. Detached sloughed tissues in the lumen were also noticed (Fig. 4B). The glomerulus showed thick corrugated capillary membrane and small less differentiated podocytes .Indentation of the nuclear membrane of the mesangial cells was seen (Fig. 4C).

Group IIb (spontaneous recovery group):

Light microscopic examination revealed that the changes in renal cortex were apparently worsened in group IIb. Shrunken glomerulus with relative widening of the capsular space and thickened parietal layer of Bowman's capsule were observed. PCTs showed hemosiderin deposition in the epithelial lining of some tubules. In DCTs there were vacuolation and shedding of tubular epithelial cells inside the lumen. Interstitial mononuclear cellular infiltrate, homogenous acidophilic hyaline casts and extensive extravasation of RBCs in the interstitium were obvious (Fig. 5A).Periodic Acid Schiff stained sections revealed thickening in parietal layer of Bowman's membrane. Negative PAS reaction of brush border of most of proximal tubular cells with PAS positive hyaline casts in the tubular lumens. Disruption of the basement membrane of some tubules could be observed (Fig. 5B). Immunohistochemical study showed increase in cytoplasmic HO−1 staining in tubules and glomeruli (Fig. 5C).

Electron microscopic examination of sections of group IIb revealed that the proximal convoluted tubule showed focal thinning of basement membrane, swollen mitochondria, degenerated areas in the cytoplasm and disrupted brush border. Hem protein deposition was noticed (Fig. 6A). Distal convoluted tubule showed condensed chromatin in the nucleus, scanty cytoplasmic organelles and the mitochondria showed partial loss of their cristae. Moreover, microvilli were lost (Fig. 6B). The glomerulus showed thick corrugated capillary membrane and small less differentiated podocytes. Shrunken nucleus of mesangial cell was seen (Fig. 6C).

Group IV (PHZ-Broccoli group):

Light microscopic examination of group IV revealed a little improvement when compared to groups IIa or IIb, as structural changes of cortical tissue were still obvious .Glomerulus retained relatively normal appearance apart from some sites of lost Bowman's space. PCTs showed hemosiderin deposition in the epithelial lining of some tubules and adhesive remnant of hyaline cast. Vacuolation, shedding of tubular epithelial cells inside the lumen and flattening of lining epithelium were detected in DCTs. Interstitial mononuclear cellular infiltrate was noticed (Fig. 7A). Periodic Acid Schiff stained sections showed that the apical brush border of some PCTs was focally interrupted. Regular basement membrane of many tubules and the parietal layer of Bowman′s capsule were noticed (Fig.7B). Immunohistochemical study revealed apparent decrease in cytoplasmic HO−1 staining in tubules and glomeruli (Fig. 7C).

Electron microscopic examination of sections of group IV showed the PCTs with intact compact brush border, large vesicular nucleus and multiple mitochondria. Hem protein deposition was still detected (Fig. 8A). DCTs showed large vesicular nucleus, mitochondria of variable size and shape, basal striation and intact dispersed microvilli (Fig. 8B). Glomerulus showed capillary membrane with relatively normal thickening and well differentiated podocytes. Dark nucleus of the mesangial cell was seen (Fig. 8C).

Group V (Broccoli-PHZ group):

Light microscopic examination of group V revealed improvement when compared to groups IIa or IIb. Glomerulus retained normal histological appearance. PCTs and DCTs were more or less relatively normal with small remnant of shedding epithelial cells inside the lumen (Fig. 9A).

Periodic Acid Schiff stained sections showed restoration of positive PAS reaction of the parietal layer of Bowman's membrane and many tubules. Most PCTs revealed PAS positive apical brush borders. Regular basement membrane of many tubules and the parietal layer of Bowman′s capsule were noticed (Fig. 9B). Immunohistochemical study showed apparent decrease in cytoplasmic HO−1 staining in tubules and glomeruli (Fig. 9C).

Electron microscopic examination of sections of group IV revealed that the PCTs showed intact compact brush border however traversed by vacuoles. The nucleus and mitochondria were relatively normal in comparison to control (Fig. 10A). The DCTs showed basal striation and intact microvilli, the cytoplasm contained a nucleus of normal pattern with mitochondria that were variable in size and shape but almost of normal size, (Fig. 10B). The Glomerulus showed capillary membrane that was relatively normal with well differentiated podocytes. Nucleus of mesangial cell of normal pattern was also noticed (Fig. 10C).

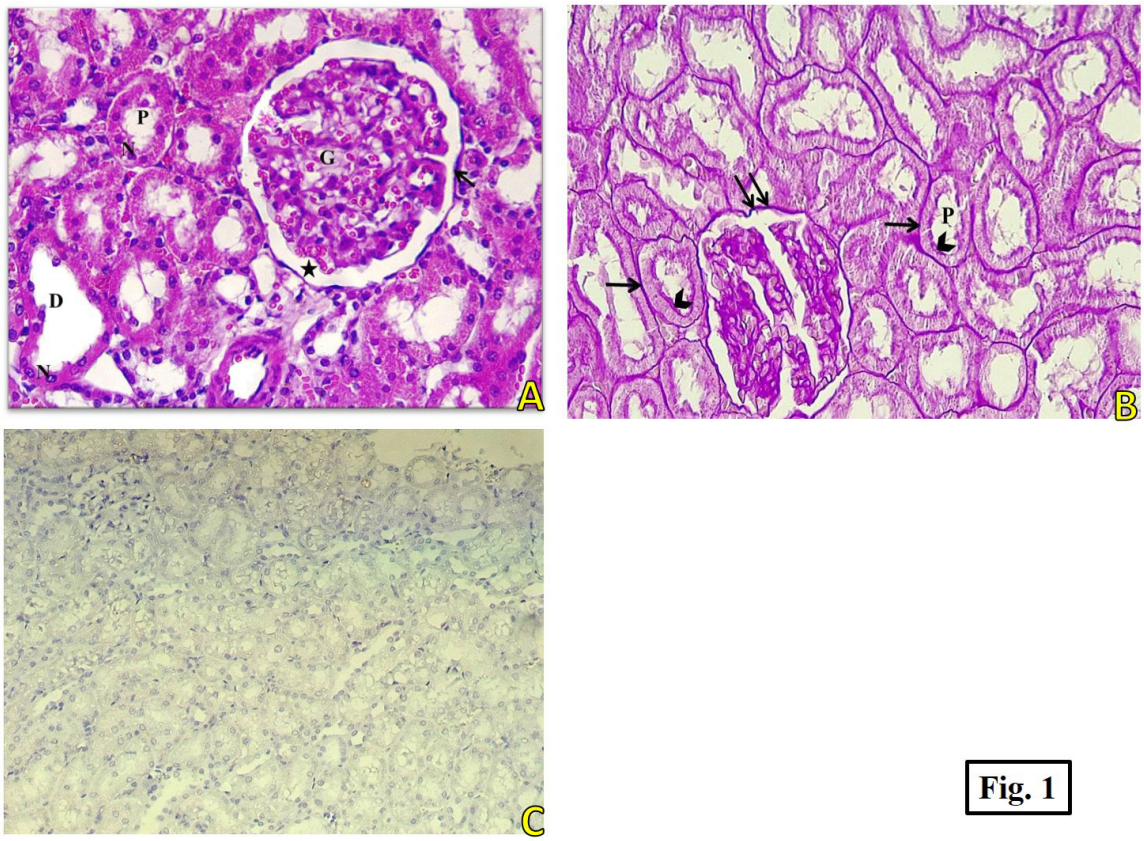


Fig. 1

Fig. 1: Photomicrographs of sections in the renal cortex of group I.
(A): showing the renal corpuscles which are formed of glomeruli (G) surrounded by Bowman's capsule (↑) and in-between is the capsular space (*). The PCTs (P) have narrow lumen and are lined with thick cuboidal epithelial cells with acidophilic cytoplasm and oval nuclei (N). The DCTs (D) have wider lumen and are lined with simple cuboidal epithelial cells with acidophilic cytoplasm and apical situated nuclei (N) (Hx& E ×400).
(B): showing the PAS positive reaction in the brush borders of the cells lining the PCTs (P) (▲), Parietal layer of Bowman's capsule (↑↑) and the basement membranes of the renal tubules (↑) (PAS x 400).
(C): showing negative cytoplasmic HO−1 staining in the tubules and glomeruli (HO−1 x200).

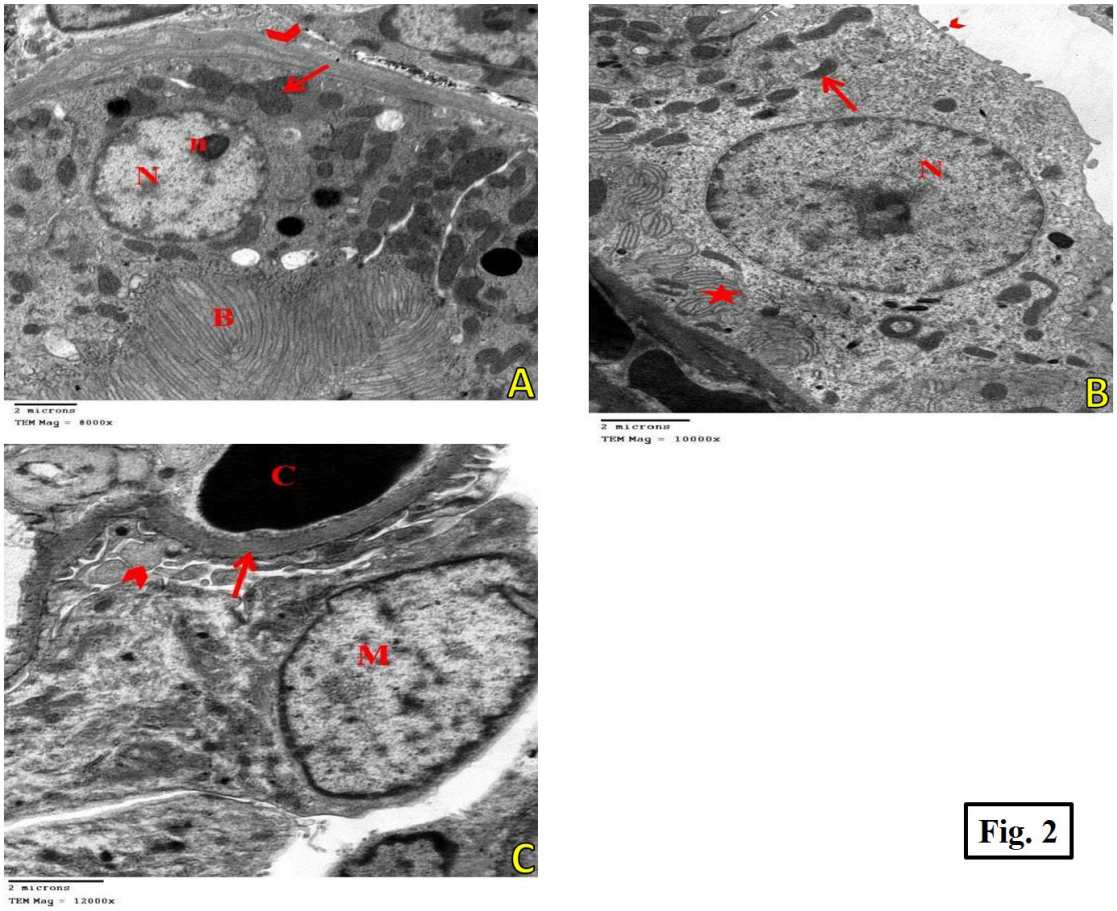


Fig. 2

Fig. 2: Transmission electron micrographs of the kidney of group I.
(A): A proximal convoluted tubule showing euchromatic nucleus (N) with prominent nucleolus (n) and numerous mitochondria (↑). Note the apical brush border (B) and multilayered basement membrane (▲) (TEM ×8000, Scale bar 2 µm).
(B): A distal convoluted tubule showing large vesicular nucleus (N), short microvilli (▲), mitochondria (↑) and endoplasmic reticulum (*) (TEM ×10000, Scale bar 2 µm).
(C): A glomerulus showing capillaries(C) With electro lucent basement membrane (↑) containing red blood cells, podocytes (▲) and mesangial cells (M) (Uranyl acetate and lead citrate ×12000, Scale bar 2 µm).

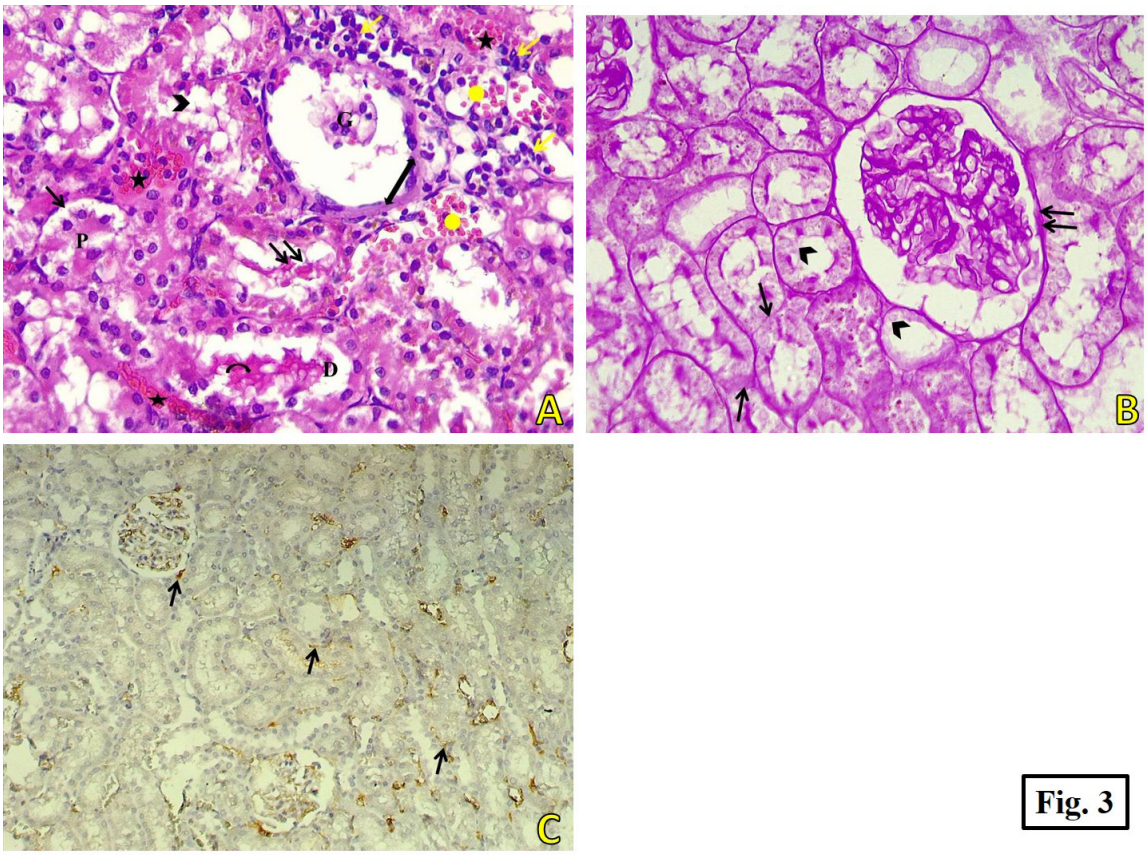


Fig. 3

Fig. 3: Photomicrographs of sections in the renal cortex of group IIa.
(A): showing shrunken glomerulus (G) with widening of capsular space and thickened parietal layer of Bowman's capsule (↔). PCTs (P) showing detachment of the epithelial lining of some tubules (↑). DCTs (D) are showing homogenous acidophilic hyaline casts in the lumen (curved arrow), vacuolation (▲) and shedding of tubular epithelial cells inside the lumen (↑↑). Interstitial mononuclear cellular infiltrate (↑) and dilated, congested peritubular capillaries (●) are seen. Extravasation of RBCs in the interstitium (*) is noticed (Hx& E ×400).
(B): showing partial loss of the brush border of the proximal tubular cells (▲). Loss of PAS reaction of many tubular basement membranes is seen (↑). Notice thickening of parietal layer of Bowman′s capsule (↑↑) (PAS x 400).
(C): showing increase in cytoplasmic HO−1 staining in tubules and glomeruli (HO−1 x200).

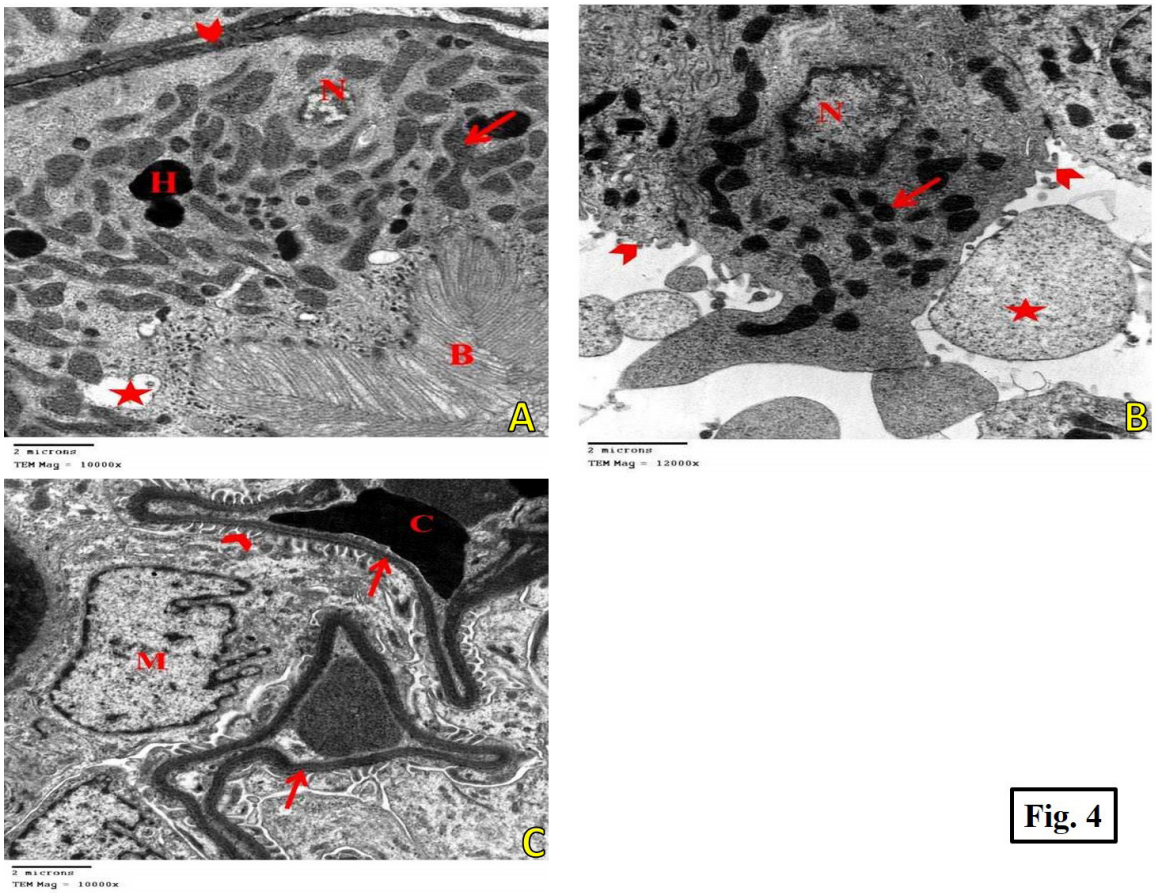


Fig. 4

Fig. 4: Transmission electron micrographs of the kidney of group IIa.
(A): A proximal convoluted tubule showing compressed basement membrane like single electron dense layer (▲), shrunken nucleus (N) and bizarre shaped mitochondria (↑).Notice the less compact brush border (B), hem protein deposition (H) and vacuoles (*) (TEM ×10000, Scale bar 2 µm).
(B): A distal convoluted tubule showing shrunken nucleus and peripheral condensed chromatin (N), multiple mitochondria (↑) and microvilli (▲). Notice the detached sloughed tissue in the lumen (*) (TEM ×12000, Scale bar 2 µm).
(C): A glomerulus showing thick corrugated capillary membrane (↑) with red blood cells in the capillaries (C) and small less differentiated podocytes (▲). Notice the Indentation of the nuclear membrane of the mesangial cells (M) (Uranyl acetate and lead citrate ×10000, Scale bar 2 µm).

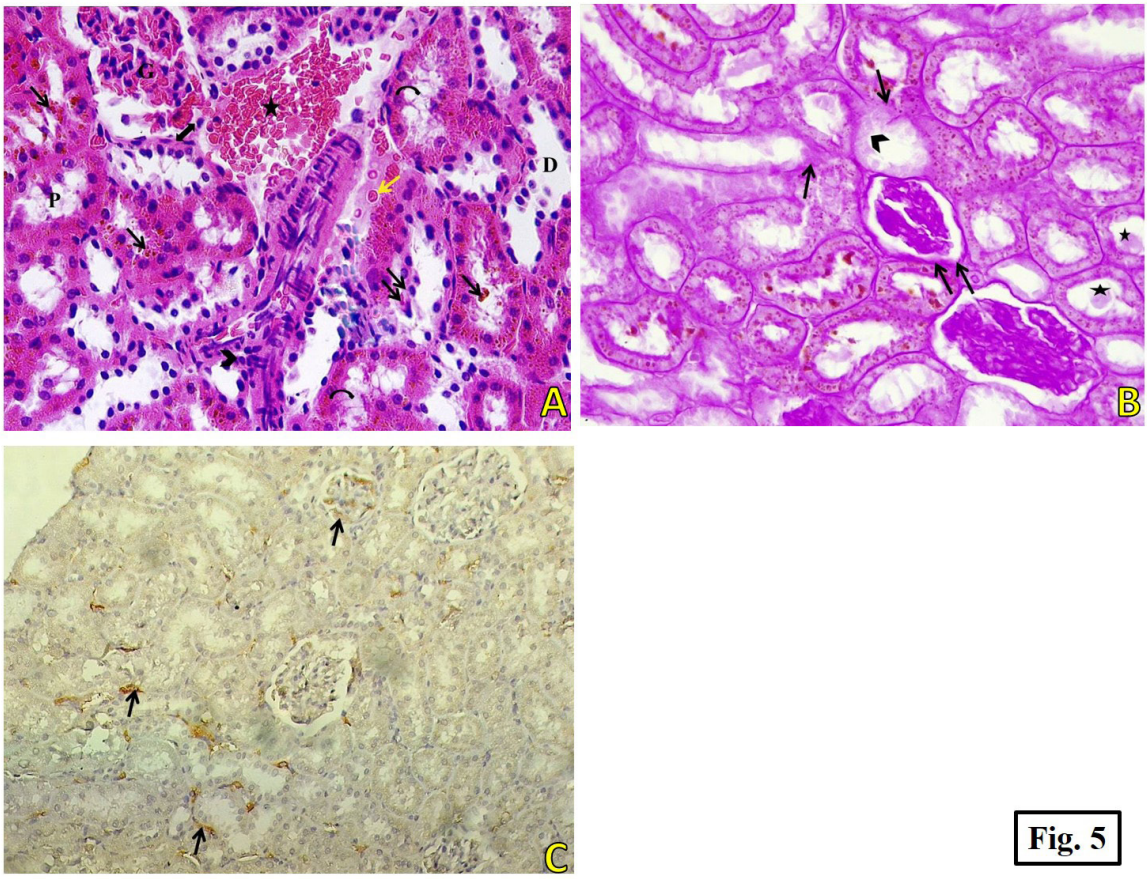


Fig. 5

Fig. 5: Photomicrographs of sections in the renal cortex of group IIb.
(A): showing shrunken glomerulus (G) with relative widening of capsular space and thickened parietal layer of Bowman's capsule (↔). PCTs (P) showing hemosiderin deposition in epithelial lining in some tubules (↑). In DCTs (D) there are vacuolation (curved arrow) and shedding of tubular epithelial cells inside the lumen (↑↑). Interstitial mononuclear cellular infiltrate (▲), homogenous acidophilic hyaline casts (↑) and extensive extravasation of RBCs in the interstitium (*) are seen (Hx& E ×400).
(B): showing thickening in parietal layer of Bowman's membrane (↑↑). Negative PAS reaction of brush border of most of proximal tubular cells (▲) with PAS positive hyaline casts in the tubular lumens (*). Disruption of the basement membrane of some tubules can be observed (↑) (PAS x 400).
(C): showing increase in cytoplasmic HO−1 staining in tubules and glomeruli (HO−1 x200).

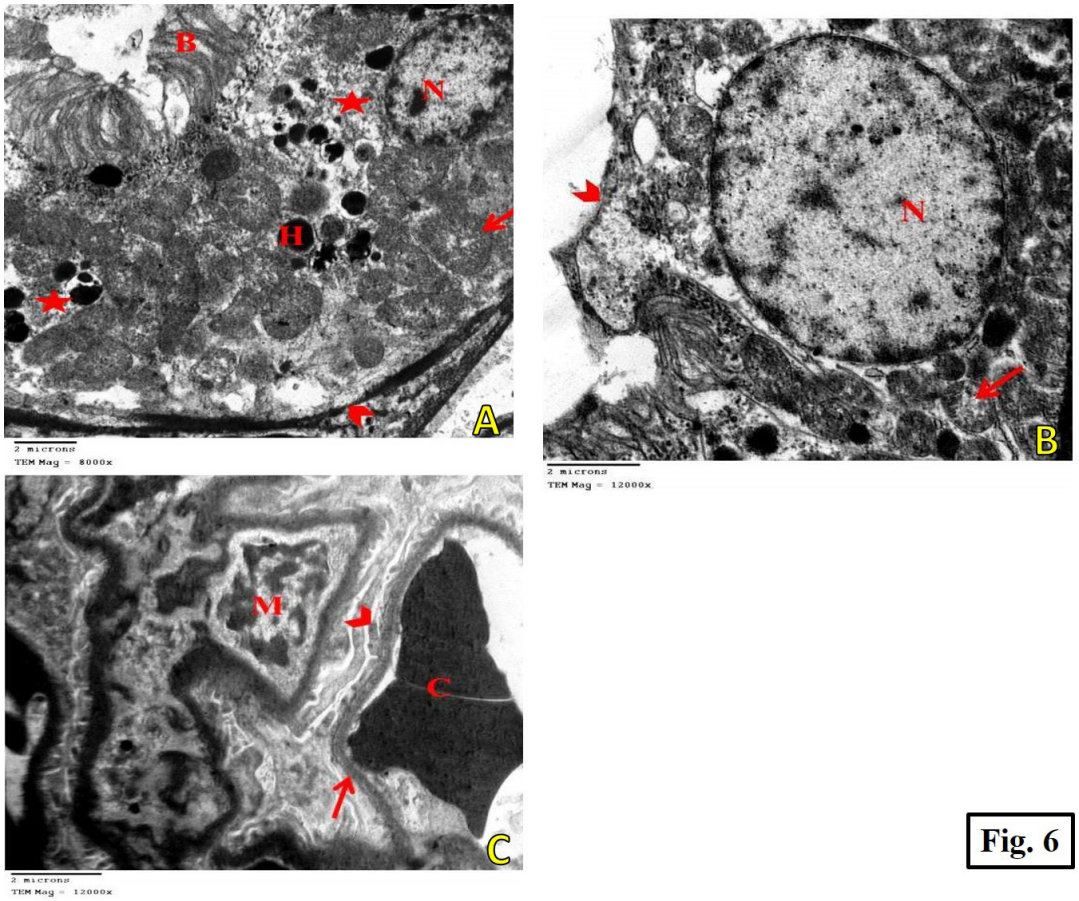


Fig. 6

Fig. 6: Transmission electron micrographs of the kidney of group IIb.
(A): A proximal convoluted tubule showing focal thinning of basement membrane (▲), nucleus (N), swollen mitochondria (↑), degenerated areas in the cytoplasm (*) and disrupted brush border (B). Notice hem protein deposition (H) (TEM ×8000, Scale bar 2 µm).
(B): A distal convoluted tubule showing condensed chromatin in the nucleus (N), mitochondria with partial loss of cristae (↑), scanty cytoplasmic organelles and lost microvilli (▲) (TEM ×12000, Scale bar 2 µm.
(C): A glomerulus showing thick corrugated capillary membrane (↑) and small less differentiated podocytes (▲). Shrunken nucleus of mesangial cell (M) is seen (Uranyl acetate and lead citrate ×12000, Scale bar 2 µm).

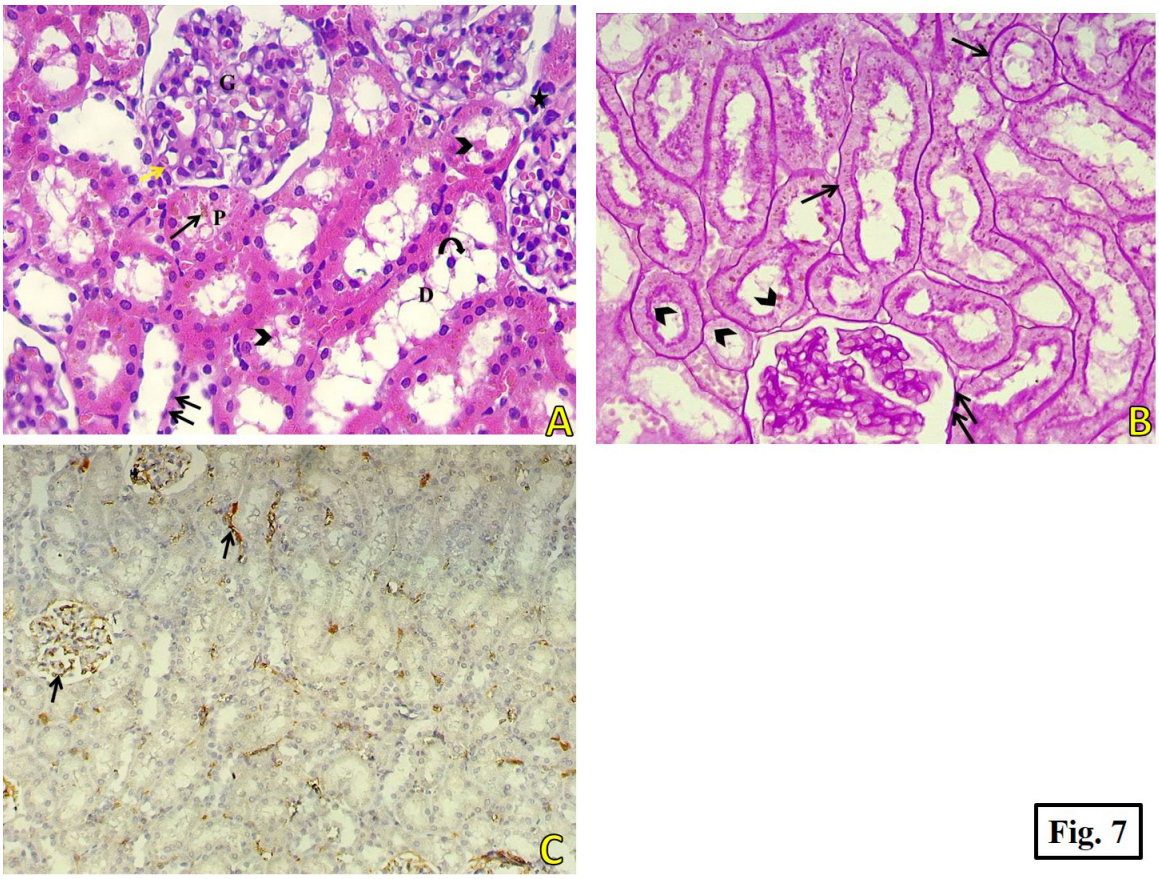


Fig. 7

Fig. 7: Photomicrographs of sections in the renal cortex of group IV.
(A): showing glomerulus (G) retained relatively normal appearance apart from some sites of lost Bowman's space (↑). PCTs (P) showing hemosiderin deposition in epithelial lining in some tubules (↑) and adhesive remnant of hyaline cast (▲). In DCTs (D) there are vacuolation, shedding of tubular epithelial cells inside the lumen (curved arrow) and flattening of lining epithelium (↑↑). Interstitial mononuclear cellular infiltrate (*) is observed (Hx& E ×400).
(B): apical brush border of some PCTs is focally interrupted (▲).Notice regular basement membrane of many tubules (↑) and the parietal layer of Bowman′s capsule (↑↑) (PAS x400).
(C): showing apparent decrease in cytoplasmic HO−1 staining in tubules and glomeruli (HO−1 x200).

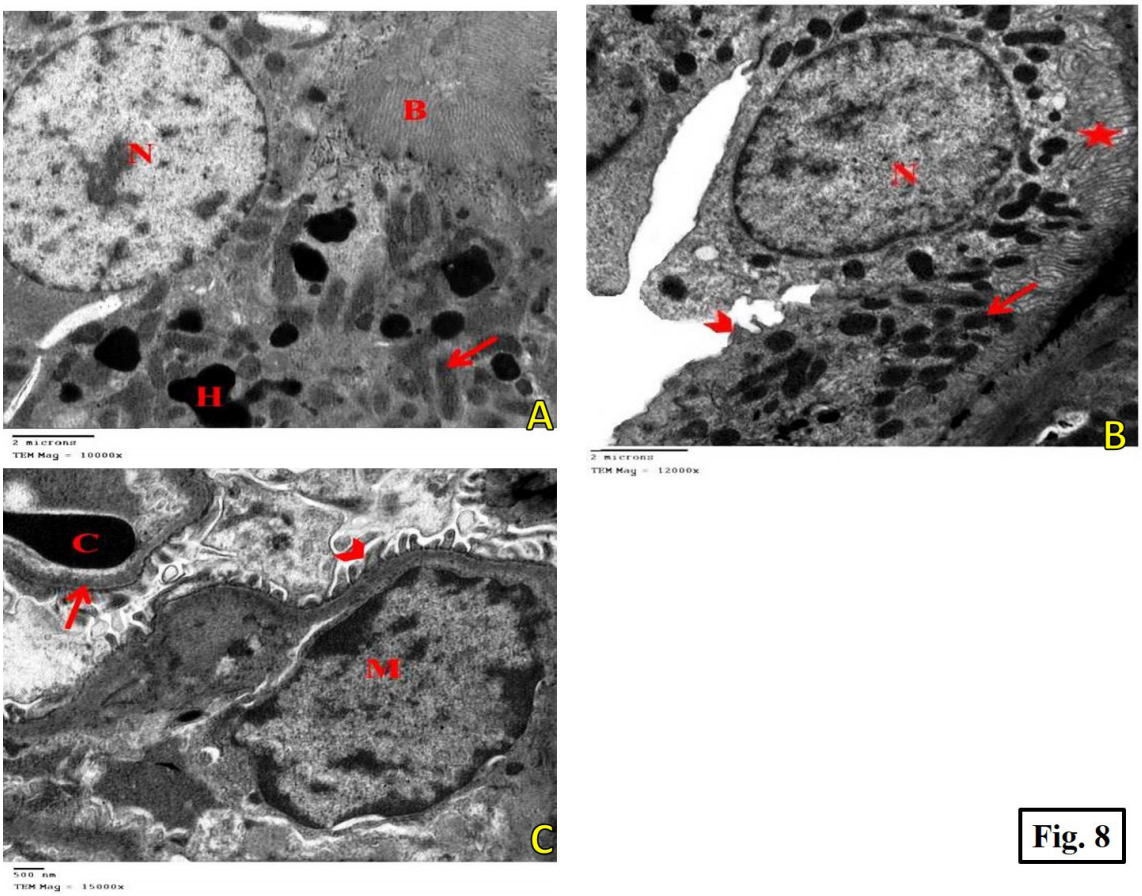


Fig. 8

Fig. 8: Transmission electron micrographs of kidney of group IV.
(A): A proximal convoluted tubule showing intact compact brush border (B) with large vesicular nucleus (N) and multiple mitochondria (↑). Notice hem protein deposition (H) (TEM ×10000, Scale bar 2 µm).
(B): A distal convoluted tubule showing large vesicular nucleus (N), mitochondria of variable size and shape (↑), basal striation (*) and intact dispersed microvilli (▲) (TEM ×12000, Scale bar 2 µm).
(C): A glomerulus showing capillary membrane with relatively normal thickening (↑) and well differentiated podocytes (▲). Notice the dark nucleus of the mesangial cell (M) (Uranyl acetate and lead citrate ×15000, Scale bar 500 nm).

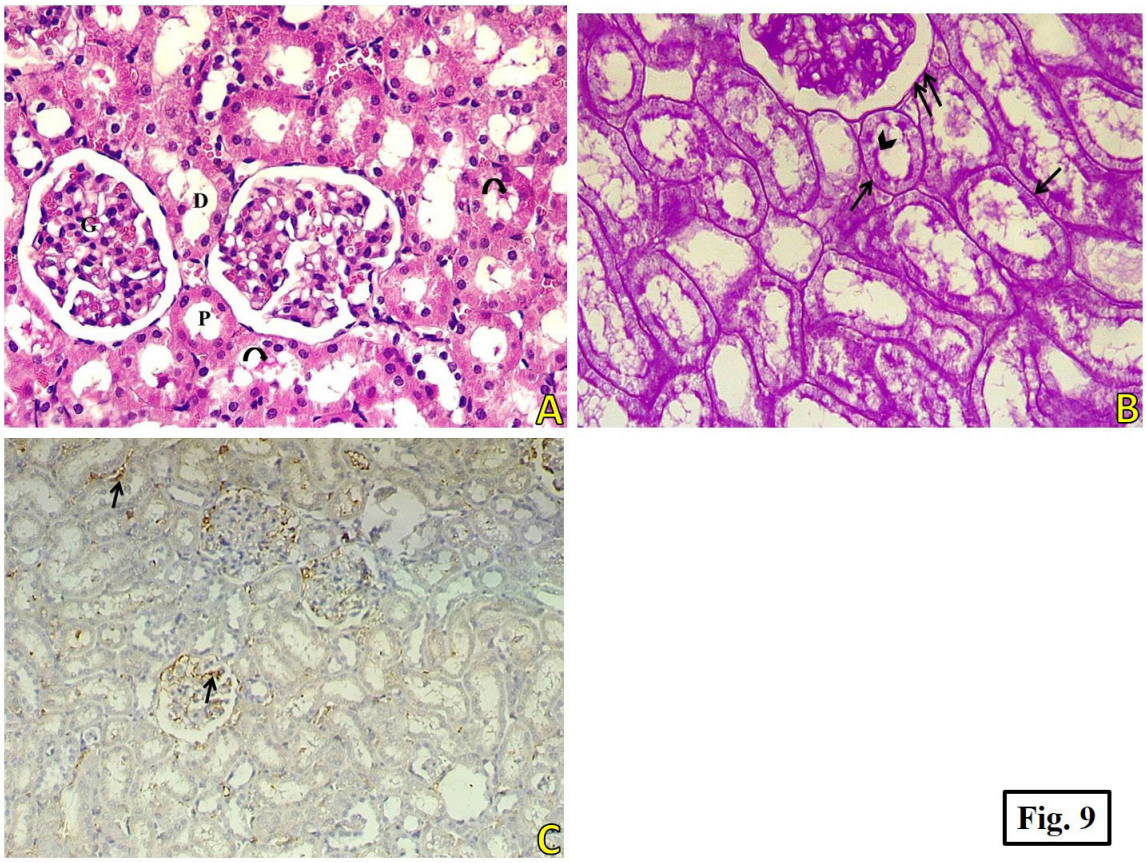


Fig. 9

Fig. 9: Photomicrographs of sections in the renal cortex of group V.
(A): showing glomerulus (G) retained normal appearance. PCTs (P) and DCTs (D) are more or less relatively normal with small remnant of shedding epithelial cells inside the lumen (curved arrow) (Hx& E ×400).
(B): showing restoration of positive PAS reaction of the parietal layer of Bowman's membrane (↑↑) and many tubules (↑). PCTs reveal PAS positive apical brush borders (▲) (PAS x400).
(C): showing apparent decrease in cytoplasmic HO−1 staining in tubules and glomeruli (HO−1 x200).

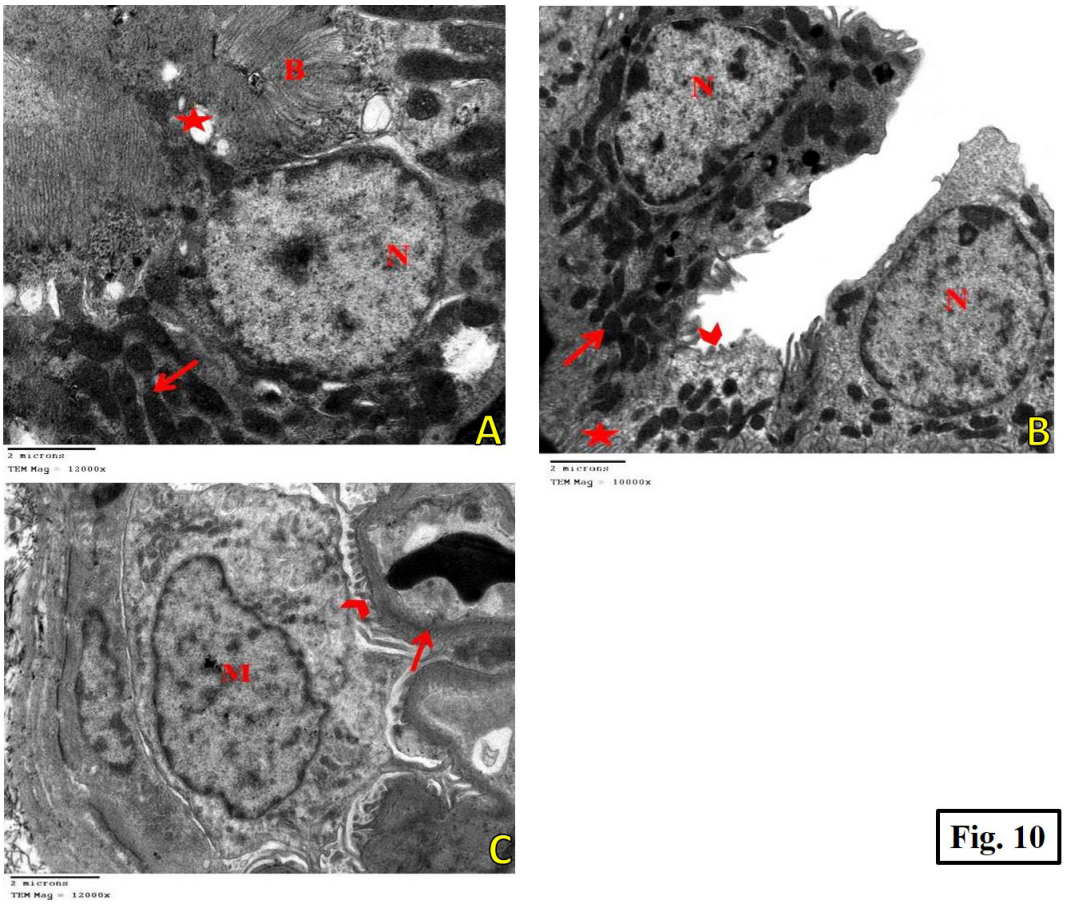


Fig. 10

Fig. 10: Transmission electron micrographs of the kidney of group V
(A): A proximal convoluted tubule showing intact compact brush border (B) however traversed by vacuoles (*). Notice the nucleus (N) and mitochondria (↑) (TEM ×12000, Scale bar 2 µm).
(B): A distal convoluted tubule showing nucleus of normal pattern (N)with mitochondria variable in size and shape almost normal size (↑), basal striation (*) and intact microvilli (▲) (TEM ×10000, Scale bar 2 µm).
(C): A glomerulus showing capillary membrane relatively normal (↑) and well differentiated podocytes (▲). Notice nucleus of mesangial cell (M) of normal pattern (Uranyl acetate and lead citrate ×12000, Scale bar 2 µm).

DISCUSSION

Hemolytic anemia has numerous causes ranging from relatively harmless to life-threatening[17]. Resultant hypoxia can cause systematic body dysfunctions[18].

Hemoglobin clearance occurred primarily through kidneys after saturation of the natural scavenging systems[19]. Normally, Hb- haptoglobin protein complexes formed in the plasma and taken up by the reticuloendothelial cells of the liver, spleen, and bone marrow in order to be degraded[3, 4, 20].

During hemolysis, in spite of hemoglobin complexes formation and degradation, free hemoglobin remains in the plasma and dissociate then filtered by the glomerular barrier[4, 5].

Substantial quantities of filtered hemoglobin are incorporated in the lumen of the tubules[21]. Also intracellular hemoglobin breaks down into haem group and free iron. This process leads to serious actions such as direct cytotoxicity, nitric oxide scavenging and vasoconstriction, inflammation, and oxidative reactions (including lipid peroxidation and mitochondrial dysfunction)[4, 22].

In the present study, PHZ used to induce hemolytic anemia. It is the most common drug used to induce experimental models of hemolytic anemia and intravascular hemolysis[5, 23].

The main action of PHZ was to induce oxidative stress within erythrocytes[24].

Oxidative stress is defined as a disruption of the prooxidant antioxidant balance. Oxidative damage to RBC's leads to formation of reactive oxygen species (ROS), premature aging of erythrocytes and predisposes to premature splenic sequestration. This leads to lack of circulating erythrocytes and hemoglobin[18, 25].

In the present study, PHZ was administrated for two days. At 4th day the hematological parameters were evaluated to confirm anemia. There were a highly statistically significant decrease in Hb, RBC's and a highly statistically significant increase in MCV, MCH. Also, there were a statistically significant decrease in PCV and statistically significant increase in WBC's.

This was in agreement with other researchers who mentioned that there were significant decrease in Hb and RBC's accompanied by significant increase in MCV, MCH and, WBC's when administrating PHZ[14, 23].

The increase in MCV was explained by the process of anucleation of erythrocyte differentiation process[26].

On the other hand, the increase in MCH is indicative of a certain degree of intravascular hemolysis and it was accompanied by a marked peripheral leukocytosis[18, 26].

Regarding kidney parameter, there were non-statistically significant increase in urea and a highly statistically significant increase in serum creatinine. This was in agreement with investigators who maintained that there were significantly increase in plasma creatinine concentration and the plasma urea in PHZ-treated mice compared with the baseline. They explained that alteration in kidney function parameter is most likely occurred due to the anemia[5].

In the current study, the histological and ultrastructural examination of renal cortex sections of group IIa revealed various structural changes in the renal cortex of PHZ treated group for a period of four days. These changes involved both the renal corpuscles and the tubules.

Glomeruli showed a histological and ultrastructure changes presented as atrophied shrunken glomeruli with subsequent widening of Bowman's space. This was coincident with 27, 28 who observed that kidney sections obtained from the PHZ treated group showed histological changes which include degeneration of the glomerulus, distention of bowman's space. They reported that PHZ exposure results in rupture of red blood cells and infiltration of the renal cortex.

In the current study, histological and ultrastructure changes were observed in the filtration barrier in the form of local thickening and corrugations of the parietal layer of Bowman's capsule. This finding was in agreement with researchers[29, 30] finding who maintained that the matrix structure of the layer altered by reactive oxygen species and became more permeable and passed more proteins than normal one.

Moreover, podocyte foot processes covering the surface were less differentiated. Researchers explained it as foot process effacement induced by oxidative stress. Also this finding explained by other investigators that this effacement represented alteration in cell to cell connections, which ranged from shortening of the processes to a complete loss of the inter-digitating foot process[31, 32].

Cytoplasmic vacuolation and shedding of the epithelial lining of the PCTs and DCTs in there lumen were noticed. These results were in agreement with investigators[24, 33] who explained that this finding occurred due to oxidative stress and the free radicals formation that facilitated the release of lysosomal enzymes into the cytosol with subsequent oxidation of the protein architecture of the cells causing cell-cell dissociation.

Moreover, 34 explained the vacuolation in the cytoplasm might be a kind of cellular defensive mechanism against injurious substances by collecting and preventing them from interfering with the biological activities of these cells.

Additionally, DCTs showed homogenous acidophilic hyaline casts in the lumen. Similar finding was reported before by 35 and explained that these luminal casts were composed of cellular fragments, sloughed epithelial cells and proteinaceous substance.

Other researchers mentioned that the intraluminal acidophilic hyaline casts formed of combination of the sloughed cells with Tamm-Horsfall protein (present in tubules lumen). Impairment of sloughed cells reabsorption by the damaged tubular cells, resulted in increased sodium concentration in the lumen and polymerization of Tamm-Horsfall protein forming a gel-like material thus contributing in cast formation[28, 36].

Additionally, interstitial mononuclear cellular infiltrate and dilated, congested peritubular capillaries were noticed in the present study. The cellular infiltration was related to oxidative stress that resulted in generation of mediators such as IL-8 and cytokine-induced neutrophil chemoattractant which attract the inflammatory cells in interstitium due to destruction of the endothelial cells by the free radicals[24, 33].

In the current study, Periodic Acid Schiff stained sections showed partial loss of the brush border of the proximal tubular cells with loss of PAS reaction of many tubular basement membranes. Ultrastructure examination revealed less compact brush border. Similarly, some investigators detected loss of the brush border, cell polarity, and adhesion between cells and the basement membrane and they explained these observations by the fact that necrosis and apoptosis can all lead to the detachment of tubular cells from the basement membrane, leaving behind areas of denuded basement membrane[28, 37].

In the present study, ultrastructure changes in the PCTs presented as shrunken nucleus, bizarre shaped mitochondria, vacuoles and hemeprotein deposition. DCTs showed shrunken nucleus, peripheral condensed chromatin and multiple mitochondria. Similar finding detected by researchers who mentioned that electron microscopy of tubules showed dark irregularly shaped granular deposits made up of heme proteins[3]. Other researchers mentioned that some cells of PCTs showed irregularly shaped nuclei, variably sized and shaped mitochondria. Cells of DCTs showed numerous variably shaped mitochondria. Some lysosomes and vacuoles were observed in both tubules[24].

Additionally, this was in agreement with other researchers who mentioned that the histologic manifestations of acute tubular injury can vary along a spectrum ranging from loss of brush border, cytoplasmic vacuolation, and cellular swelling to extensive necrosis of tubular cells[38)].

In the present study, immunohistochemical study showed increase in cytoplasmic HO−1 staining in tubules and glomeruli. This is in agreement with other investigators who mentioned that HO-1 is responsible for heme degradation, and protection against oxidative stress[39]. PHZ treatment induced upregulation of selective HO-1 production within these cells will play an important protective role in preserving renal functions[40].

In the current study, examination of histological sections of group IIb (spontaneous recovery group) revealed apparent worsening of the structural changes in comparison to group IIa and appearance of hemosiderin deposition in epithelial lining in some tubules. This was in agreement with other researchers who maintained that hemosiderin deposition in the renal tubular cells is the most consistent finding in all patients with intravascular hemolysis[38].

In spite of that there were statistically significant improvement in Hb, RBC's, WBC's and urea and non-statistically significant improvement in PCV, MCV, MCH and serum creatinine. This statistical finding was in agreement with other investigators who maintained that the regenerative process of erythropoietic system following PHZ-induced hemolytic anemia is time and dose-dependent[26].

Antioxidants are responsible for control oxidative damage done to red cells by free radicals or highly reactive oxygen species[12].

Broccoli is a rich source of minerals and vitamins which have antioxidant and antiproliferative activity and may help in haemoglobin formation[11, 41].

Additionally, investigators proved that aqueous extract of Broccoli have a protective role in PHZ induced anemia as it's contain powerful antioxidants called alkaloids which prevent cell damage by free radicals[10, 42].

Researchers found an alternative way to utilize broccoli crop through drying of it and use the powder. The powder contains a good amount

of nutritional composition, dietary fibers and few amounts of sugar especially in floret[11].

Also, other investigators proved that broccoli powder results in a significant increase in glutathione S-transferase enzyme (GST) concentration in kidney tissues of diabetic rats[43].

In the present study, examination of stained renal cortical sections of group IV that received curative treatment with broccoli revealed a little improvement of the histopathological and ultrastructure changes caused by hemolytic anemia as there is still hemosiderin deposition in the epithelial lining of PCTs with adhesive remnant of hyaline casts and focally interrupted brush border by PAS stain. DCTs showed vacuolation and shedding of epithelium lining with interstitial mononuclear infiltration. Immunohistochemical staining showed apparent decrease in cytoplasmic HO−1 staining in tubules and glomeruli.

Moreover, regarding statistical results revealed a highly statistically significant increase in Hb, MCV and urea. RBC's, WBC's and serum creatinine showed statistically significant difference and non-statistically significant change in PCV and MCH. This was in agreement with other investigators[10].

In the current study, administration of broccoli as a prophylactic treatment in group V revealed marked improvement of the histological structure and ultrastructure of renal cortex. Examination of group V sections showed restoration of the architecture of renal corpuscles, PCTs and DCTs as compared to the control group. By PAS stain, broccoli had markedly preserved the normal thickness of parietal layer of Bowman's capsule and many tubules. PCTs restored their PAS positive apical brush borders. Immunohistochemical staining showed apparent decrease in cytoplasmic HO−1 staining in tubules and glomeruli.

Researchers' maintained effectiveness of broccoli as a promising curative treatment for the kidney in case of induced diabetes through its antioxidant activity[12, 13, 41, 43].

Additionally, regarding statistical results revealed there is a highly statistically significant increase in RBC's, statistically significant difference in Hb, PCV, WBC's, MCV, MCH and serum creatinine and non-statistically significant decrease in urea.

CONCLUSIONS

From the present study, it can be concluded Broccoli extract has antianemic, antioxidant activity as prophylactic treatment for patient with hemolytic anemia with good kidney function better than curative treatment after kidney injury. It is recommended for patients with hemolytic anemia to eat Broccoli sprout to protect their kidneys.

CONFLICT OF INTERESTS

There are no conflicts of interest.

REFERENCES

1. Onyeabo C, Achi NK, Ekeleme-Egedigwe CA, Ebere CU and Okoro CK. HAEMATOLOGICAL AND BIOCHEMICAL STUDIESON JUSTICIA CARNEA LEAVES EXTRACT IN PHENYLHYDRAZINE INDUCED-ANEMIA IN ALBINO RATS. Acta Sci. Pol. Technol. Aliment. 2017; 16(2): 217–230.

2. Kushwah C, Gupta D, Joshi A, Malviya S, Kharia A. Anti-Anemic Activity of Hydro-Alcoholic Extract fruit of Physalis minima in Phenylhydrazine Induced Anemic Rats. International Journal of Research 201; 05(12): 3618-3620.

3. Qian Q, Nath KA, Wu Y, Daoud TM, Sethi S. Hemolysis and Acute Kidney Failure. Am J Kidney Dis. 2010; 56(4): 780–784.

4. Guerrero-Hue M, Rubio-Navarro A, Sevillano Á, Yuste C, Gutiérrez E, Palomino-Antolín A, Román E, Praga M, Egido J, Moreno JA. Adverse effects of the renal accumulation of haem proteins. Novel therapeutic approaches. Nefrologia. 2018; 38(1):13-26.

5. Merle NS, Grunenwald A, Figueres M-L, Chauvet S, Daugan M, Knockaert S, Robe-Rybkine T, Noe R, May O, Frimat M, Brinkman N, Gentinetta T, Miescher S, Houillier P, Legros V, Gonnet F, Blanc-Brude OP, Rabant M, Daniel R, Dimitrov JD, Roumenina LT. Characterization of Renal

Injury and Inflammation in an Experimental Model of Intravascular Hemolysis. Front. Immunol. 2018; 9:179.

6. Adibmoradi G. Effect of phenylhydrazine-induced hemolytic anemia on morphological changes of renal cortex in adult mice. Journal of Veterinary Science & Technology2017; 8:5

7. Rahman A, Yamazaki D, Sufiun A, Kitada K, Hitomi H, Nakano D, Nishiyama A . A novel approach to adenine-induced chronic kidney disease associated anemia in rodents. PLoS ONE 2018; 13(2): e0192531.

8. Shukla P, Yadav NK, Singh P, Bansode FW, Singh RK. 2012. PHENYLHYDRAZINE INDUCED TOXICITY: A REVIEW ON ITS HAEMATOTOXICITY. International Journal of Basic and Applied Medical Sciences 2012; 2(2): 86-91

9. Anbara H, Shahrooz R, Razi M, Malekinejad H, Najafi GhR. The effect of vitamin C on mice hemolytic anemia induced by phenylhydrazine: an animal model study using histological changes in testis, pre-implantation embryo development, and biochemical changes. Iran J Basic Med Sci. 2018; 21(7):668-677.

10. Avula VV, Bora D, Upreti S, Jonathan SK, Rijal S. EFFECT OF AQUEOUS EXTRACT OF BRASSICA OLERACEAE VAR ITALICA (BROCCOLI) INFLORESCENCE IN PHENYLHYDRAZINE INDUCED ANEMIC RATS. Bulletin of Pharmaceutical Research 2015; 5(3):81-83.

11. Madhu, Kochhar A. Proximate composition, available carbohydrates, dietary fibre and anti-nutritional factors of Broccoli (Brassica oleracea l var. Italica plenca) leaf and floret powder. Bioscience Discovery 2014; 5(1): 45-49.

12. Suresh S, Waly MI, Rahman MS, Guizani N, Al-Kindi MAB, Al-Issaei HKA, Al-Maskari SNM, Al-Ruqaishi BRS, Al-Salami A. Broccoli (Brassica oleracea) Reduces Oxidative Damage to Pancreatic Tissue and Combats Hyperglycaemia in Diabetic Rats. Prev Nutr Food Sci. 2017; 22(4): 277–284.

13. Patel V, Sharma V. Effect of Brassica oleracea Extracts on Blood Glucose and Antioxidant Profile in Streptozotocin Induced Diabetic Rats. Journal of Medical and Pharmaceutical Innovation 2014; 1(5): 4-9.

14. Lee HW, Kim H, Ryuk JA, Kil KJ, Ko BS. Hemopoietic effect of extracts from constituent herbal medicines of Samul-tang on phenylhydrazine-induced hemolytic anemia in rats. Int J Clin Exp Pathol. 2014; 7(9): 6179–6185.

15. Bancroft J, Gamble M. Marsland, Glees and Erikson's techniques: in theory and practice of histological techniques. 5th ed. Churchill livingstone, London 2002; 104-106.

16. Bancroft JD, Gamble M. 2008. Theory and practice of histological techniques (6th ed.). Philadelphia, PA: Churchill Livingstone. 2008.

17. Ashour TH. Hematinic and anti-anemic effect of thymoquinone against phenylhydrazine-induced hemolytic anemia in rats. Research Journal of Medical Sciences 2014; 8(2): 67-72.

18. Aduwamai UH, Abimbola MM, Ahmed ZH. Effect of Solanum nigrum Methanol Leaf Extract on Phenylhydrazine Induced Anemia in Rats. Jordan Journal of Biological Sciences 2018; 11(11): 65-71.

19. Abd El Zaher F, El Shawarby A, Hammouda G, Bahaa N. Role of Mesenchymal Stem Cells versus their Conditioned Medium on Cisplatin-Induced Acute Kidney Injury in Albino Rat. A Histological and Immunohistochemical Study. The Egyptian Journal of Histology 2017; 40(1): 37-51.

20. Fervenza FC, Croatt AJ, Bittar CM, Rosenthal DW, Lager DJ, Leung N, Zeldenrust SR, Nath KA. Induction of Heme Oxygenase-1 and Ferritin in the Kidney in Warm Antibody Hemolytic Anemia. Am J Kidney Dis. 2008; 52(5): 972–977.

21. Tracz MJ, Alam J, Nath KA. Physiology and pathophysiology of heme: implications for kidney disease. J Am Soc Nephrol. 2007;18: 414–420.

22. Vasconcellos LR, Dutra FF, Siqueira MS, Paula-Neto HA, Dahan J, Kiarely E, Carneiro LA, Bozza MT, Travassos LH. Protein aggregation as a cellular response to oxidative stress induced by heme and iron. Proc Natl Acad Sci U S A. 2016; 113(47):E7474-E7482.

23. Pingali PS, Srinivas P, Reddy BM. 2015. STUDY OF ANTI-ANAEMIC EFFECT OF SCHREBERASWIETENIOIDES ROXB. IN RAT MODELS. Asian Journal of Pharmaceutical and Clinical Research 2015; 8(5):305-308.

24. Elwan WM, Ragab AMH, Ragab MH. Histological and immunohistochemical evaluation of the dose-dependent effect of gold nanoparticles on the renal cortex of adult female albino rat. The Egyptian Journal of Histology 2018; 41(2): 167-181.

25. Jaiswal A, Ganeshpurkar A, Awasthi A, Bansal D, Dubey N. Protective Effects of Beetroot Extract against Phenyl Hydrazine Induced Anemia in Rats. Pharmacognosy Journal 2014; 6(5): 1-4.

26. Roque M, D´Anna C, Gatti C, VeutheyT. Hematological and Morphological Analysis of the Erythropoietic Regenerative Response in Phenylhydrazine-induced Hemolytic Anemia in Mice. Scand. J. Lab. Anim. 2008; 35(3): 181-190.

27. Hamman L L, Amaza DS, Zirahei1 JV, Goji ADT, Mari H, Amali F. EFFECT OF AQUEOUS EXTRACT OF BITTER LEAF (VERNONIA AMYGDALINA) ON PHENYL-HYDRAZINE INDUCED KIDNEY DAMAGE IN ALBINO RAT. Int. J. Adv. Res.2016; 4(11): 39-47.

28. Sakr AI, Abd Elhai WM, Abo Zeid AA, Ali HE. Transplanted Adipose Derived Mesenchymal Stem Cells Attenuate The Acute Renal Injury Induced by Cisplatin in Rats. The Egyptian Journal of Histology 2017; 40(2): 169-183.

29. Eid RA. Apoptosis of Rat Renal Cells by Organophosphate Pesticide, Quinalphos: Ultrastructural Study. Saudi J Kidney Dis Transpl. 2017; 28(4):725-736.

30. Elsaid AG, Faheem NM. Alkaline Ionized Water Ameliorates the Structural and Ultrastructural Changes Induced by Hyperlipidemia on the Renal Cortex of the Adult Albino Rats. Egyptian Journal of Anatomy 2017; 40(1):189-202.

31. Abdel-Hafez AMM, Othman MA, Seleim MAA. Effect of shark liver oil on renal cortical structure in hypercholesterolemic rats. The Egyptian Journal of Histology 2011; 34(2):391–402.

32. Mohamed HK, Meligy FY. The possible protective Effects of Alfa Lipoic Acid on Diethanolamine-Induced Renal Toxicity in Adult Male Albino Rats: A Histological and Immunohistochemical Study. The Egyptian Journal of Histology 2018; 41(4): 431-444.

33. Ahmed SM, Mohammed MZ, Mahmoud AA. The role of gold nanoparticles on taxol-induced renal cortex damage in adult male albino rats. The Egyptian Journal of Histology 2018; 41(3): 237-249.

34. Ragab EE. The possible protective effect of vitamin C on monosodium glutamate induced renal toxicity in male albino rats. The Egyptian Journal of Histology 2018; 41(4): 386-397.

35. El-Hawwary AA, Sarhan NR. Does Olive Oil Attenuate Ciprofloxacin-Induced Renal Cortical Toxicity? Light and Electron Microscopic Study. The Egyptian Journal of Histology 2017; 40(2): 253-264.

36. Abuelo JG. Normotensive ischemic acute renal failure. N Engl J Med.2007; 357(8): 797- 805.

37. Ezz-Din D, Gabry MS, Farrag AR, Abdel -Moneim AE. Physiological and histological impact of Azadirachta indica (neem) leaves extract in a rat model of cisplatin-induced hepato and nephrotoxicity. J Med Plants Res. 2011; 5(23): 5499 -5506.

38. Puri V, Gandhi A, Sharma S. Renal biopsy in paroxysmal nocturnal hemoglobinuria: An insight into the spectrum of morphologic changes. Indian J Nephrol. 2017; 27(4): 284-288.

39. Olsson MG, Allhorn M, Bülow L, Hansson SR, Ley D, Olsson ML, Schmidtchen A, Akerström B. Pathological conditions involving extracellular hemoglobin: molecular mechanisms, clinical significance, and novel therapeutic opportunities for α (1) microglobulin. Antioxid Redox Signal. 2012; 17(5):813-846.

40. Morimoto K, Ohta K, Yachie A, Yang Y, Shimizu M, Goto C, Toma T, Kasahara Y, Yokoyama H, Miyata T, Seki H and Koizumi S. Cytoprotective role of heme oxygenase (HO)-1 in human kidney with various renal diseases. Kidney Int. 2001; 60(5):1858-66.

41. Bachiega P, Salgado JM, de Carvalho JE, Ruiz ALTG, Schwarz K, Tezotto T, Morzelle MC. Antioxidant and antiproliferative activities in different maturation stages of broccoli (Brassica oleracea Italica) biofortified with selenium. Food Chemistry 2016; 190: 771–776.

42. Ogbe RJ, Adoga GI, Abu AH. Antianaemic potentials of some plants extracts on phenyl hydrazine induced anaemia in rabbits. J. Med. Plants Res. 2010; 4(8):680-684.

43. El Hendy HA, Al-Gemeai ARO. Effect of broccoli intake on antioxidant in the liver and kidney tissues of hyperglycemic rats. Integr Food Nutr Metab. 2014; 1(1): 83-86.

مستخلص البروكلي يحمي من تلف القشرة الكلوية المستحدث بواسطة فقر الدم الانحلالي في ذكور الجرذان البالغة

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ملخص البحث

مقدمة: فقر الدم الانحلالي يتسبب في اختلالات وظيفية في الجسم. يتم إزالة الهيموغلوبين عن طريق الكلى بعد تشبع أنظمة الكسح الطبيعية. مستخلص البروكلي فعال في علاج العديد من الأمراض كمادة مضادة للأكسدة.

الهدف: تهدف هذه الدراسة الي تقييم التأثير الوقائي لمستخلص البروكلي على تلف القشرة الكلوية الناجم عن فقر الدم الانحلالي.

المواد والطرق: ثماني وأربعون من الجرذان الذكور البيضاء البالغة استخدمت في هذه الدراسة وتم تقسيمهم الي خمس مجموعات. المجموعة الأولي: كمجموعة ضابطة، المجموعة الثانية: تحتوي على أثني عشر جرذا تلقت حقنة واحدة بالغشاء البريتوني ٦٠ ملج/كج من الفينيل هيدرازين ثم تم تقسيمها الي مجموعتين متساويتين. المجموعة الثالثة: ست من الجرذان تلقت ٢٠٠ ملج/كج من مستخلص البروكلي عن طريق الفم. المجموعة الرابعة: ست من الجرذان تم حقنها بالفينيل هيدرازين ثم تلقت مستخلص البروكلي بنفس الجرعة وطريقة المجموعات السابقة. المجموعة الخامسة: ست من الجرذان تلقت البروكلي ثم تم حقنها بالفينيل هيدرازين بنفس الجرعة وطريقة المجموعات السابقة. في نهاية التجربة، تم سحب عينات الدم وأخذ عينات الكلي وتحضيرها للفحص بالمجهر الضوئي والالكتروني.

النتائج: أظهر فحص القشرة الكلوية للمجموعة الثانية أ انكماش بالكبيبة، زيادة في سمك الطبقة الجدارية لكبسولة بومان، احتقان بالأوعية الدموية والتسلل الخلوي وبالفحص المجهري الإلكتروني وجد تشوه بالقشرة الكلوية. من ناحية أخري، أظهر فحص المجموعة الرابعة والخامسة تعديلًا واضحًا في جميع الطبقات.

الاستنتاج: لمستخلص البروكلي تأثير وقائي أفضل من الدور العلاجي ضد تلف قشرة الكلى الناجم عن فقر الدم الانحلالي.