Original Article

A Comparative Hematological, Biochemical and Renal Histological Study of *Gerbillus pyramidum*, *Mus musculus* and *Rattus norvegicus* from Three Different Regions in Egypt

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ABSTRACT

Introduction: Rodents are the best-studying mammalian species in terms of classification and biology (histological structure and physiology). Gerbils, mice, and rats are the favorite mammal species for most studies due to their small size, short lifespan, and rapid reproduction.

Aim of the Work: We tried to demonstrate the kidney histological structure and functions, oxidative stress, and immunohistochemical expression of *Gerbillus pyramidum*, *Mus musculus*, and *Rattus norvegicus* collected from three different habitats.

Materials and Methods: We collected 21 live specimens; 7 of G. *pyramidum* from the New Valley region, 8 of M. *musculus* from the Faiyum region, and 6 of R. *norvegicus* from the Eastern Desert region.

Results: *G. pyramidum* recorded six significantly increased biochemical parameters which are ALT, AST, cholesterol, triglycerides, sodium, and chloride. Also, *M. musculus* recorded six significant increase in the values of urea, creatinine, uric acid, alkaline phosphatase, calcium–ionized, and phosphorous. Total protein, potassium, calcium, and magnesium were the four biochemical parameters recorded as significant increase for *R. norvegicus*. The results confirmed that the New Valley region gerbils, who live in a desert arid area, have kidneys that are more efficient than those that live in Faiyum and Eastern Desert regions. The greater relative medullary thickness 2.83±0.45 was recorded for New Valley gerbils and the lowest one was 2.15±0.50 for Faiyum mice. The average juxtamedullary glomeruli diameter was greater 1.63 times than superficial glomeruli of *G. pyramidum*, 1.45 times of M. musculus, and 1.29 times of *R. norvegicus*. The highest values of the kidney GSH, SOD, and CAT were recorded for *G. pyramidum*, and the minimum values of the kidney GSH, SOD, and CAT were recorded for *M. musculus*. Weak expression of both anti-apoptotic protein bcl2 and pro-apoptotic protein p53 was observed for rodents' kidney tissues from New Valley and Eastern Desert Regions but the Faiyum mice recorded positive strong immunoreactivity of bcl2 and moderate positive reactivity of p53 for cortex and strong expression for medulla of kidney tissues.

Conclusion: *G. pyramidum* from the New Valley region has the highest RMT, superficial glomerular number, diameter, volume, RGBV, juxtamedullary glomerular number, and RGBV than other studied rodents.

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Key Words: Eastern desert, faiyum, histological measurements, new valley.

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INTRODUCTION

The world's deserts are subsisting of a variety of rodent species. Rodents in the desert can survive for long times without drinking water. These animals obtain water either by feeding, metabolically, or by reducing water loss by avoiding exposure to high temperatures in the daytime, regulating their respiration, and concentrating urine[1]. In arid areas, rodents manage and regulate their water balance through several physiological mechanisms and changes in their kidney functions[2] and ecological factors[3] or increases in plasma and pituitary anti-diuretic hormones[4].

Desert rodents protect themselves from climate variability by living in relatively deep burrows. They are adapted to the amount of food it finds in their habitat. Water metabolism in desert rodents is highly diversified according to species and is related to many criteria such as geographical location, season, and climatic factors reflecting the various adaptation strategies[5].

The desert rodents' physiological adaptation involved their ability to reduce water removal by concentrating urine[6]. They also have a food selection (behavioral adaptation) that can be important for water conservation. The desert rodents have a lower water consumption of about 5% to maintain their water balance[7].

In all animals, the kidney is a very important organ of the urinary system. The kidney is responsible for the maintenance of homeostasis through several complex processes started with the filtration of blood, absorption of some useful substances and expel of other harmful substances. The mammalian kidney also regulates the fluid and electrolyte balance of the body. It has sites for renin production and helps in blood pressure regulation[8].

The structural and functional unit of the mammalian kidney mainly consists of a nephron and its collecting ducts. The nephron is divided into several distinct parts which are the Malpighian corpuscle, proximal convoluted tubule, Henle's loop, distal convoluted tubule, and

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collecting tubules[9]. A typical bean-shaped appearance is the main characteristic of the mammalian kidney[10]. The rodent kidneys are unipapillary and mainly formed of the outer capsule, renal cortex, and medulla. The renal cortex is highly vascular and the medulla is slightly thicker than the cortex and less vascular[11]. This work aimed to demonstrate the kidney histological structure and functions, oxidative stress, and immunohistochemical expression of *G. pyramidum*, *Mus musculus*, and *Rattus norvegicus* collected from three different habitats which are New Valley, Faiyum Depression, and Eastern Desert of Egypt, respectively.

MATERIALS AND METHODS

The collected specimens

A total of 21 specimens from both sexes were captured. Seven specimens of the greater Egyptian gerbil, *Gerbillus pyramidum* were collected from New Valley (near Baris Village, Kharga Oasis), eight specimens of the house mouse, *Mus musculus* from Faiyum (Al Idwah houses adjacent agricultural fields), and six specimens of Norway rat, *Rattus norvegicus* from Eastern Desert (near Hurghada Airport) (Figure 1).

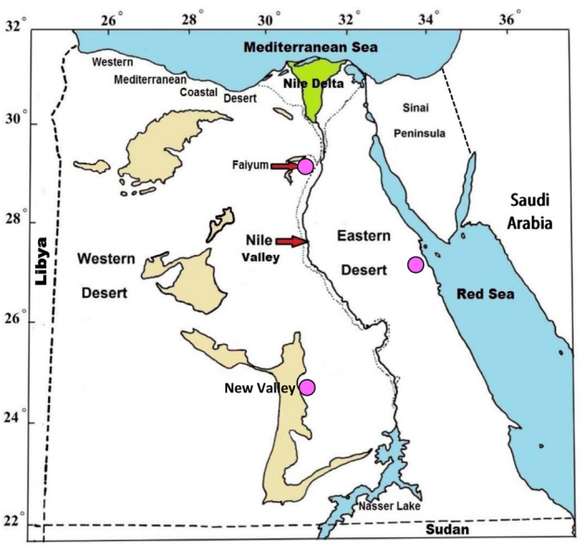

Fig. 1: Map of Egypt showing the three study sites, New Valley, Faiyum and Eastern Desert (pink circles).

Examination of blood samples

The blood serum was separated from each tube after the blood samples were taken from the studied rodents. About one cm3 of the blood sample was mixed with EDTA for measuring the hematological parameters. The biochemical parameters were measured from the separated blood sera. After dissecting the studied rodents, we gently remove and weigh their kidneys. To calculate the kidney-to-body ratio (KBR), the kidneys of each rodent were weighed. KBR equals kidney weight in grams x 100/ rodent body weight in grams. After 24 hours in Bouin's fixation, one kidney of each rodent was embedded in paraffin wax for light microscopy examination. Samples of the kidney were placed in 10% neutral buffered formalin for immunohistochemical investigation. For determination of antioxidant enzyme activity, the other rodent kidney was homogenized in 5% phosphate buffer solution pH 7.4 then centrifuged for 15 minutes at 3000 rpm and the supernatant was gently taken and stored at -20°C until processing.

Hematological and biochemical parameters

We examined the blood samples for counting RBCs, WBCs, and platelets using Neubauer counting hemocytometer. Hemoglobin (catalogue number 610001), hematocrit, MCV, MCH, MCHC, and differential leucocytes also were determined. The blood count methods were assayed using commercial kits supplied by Spectrum Company.

Enzymatically, the blood urea (catalogue number U119240) was estimated according to the method of Tietz[12] using BIOMED Diagnostics Company kit. Uric acid (catalogue number UA119090) and creatinine (catalogue number CRE105100) were estimated after Tietz[13] using BIOMED Diagnostics Company kits. Using Spectrum Company kits, the activities of aspartate aminotransferase (catalogue number 291007) and alanine aminotransferase (catalogue number 263003) in serum were measured kinetically. Total protein (catalogue number 213001), serum albumin (catalogue number 211001), and alkaline phosphatase (catalogue number 216001) were assessed using commercial kits supplied by Spectrum Company. Serum cholesterol (catalogue number 1001192), triglycerides (catalogue number 1001191) were assessed using commercial kits supplied by Spinreact Company kits. Chloride (catalogue number 233001), sodium (catalogue number 303002), potassium (catalogue number 298002), calcium (total and ionized) (catalogue numbers 226002 and 227001, respectively), and phosphorous (catalogue number 296001) values were measured using Spectrum Company kits. Magnesium (catalogue number MG122120) were measured using BIOMED Diagnostics Company kit.

Histological observations

The Bouin's fixed kidneys were carefully sectioned at 5 µm by laboratory rotational microtome. The stained sections with hematoxylin and eosin were used for histological investigation[14].

Measuring the different kidney parameters

The renal cortical, outer and inner medullary thickness were measured using a calibrated eyepiece. The total, superficial, and juxtamedullary glomerular numbers in a longitudinal section were counted. Using the formula $GV = \pi \div 6 \times (LB)^{1.5}$, the glomerular volume was calculated, where π is a constant equal to 3.14, L is the long glomerular axis and B is the short axis of the glomerulus. The diameter of the glomerulus was calculated as GD = (L+B) ÷ 2. Using the formula RGBV = GV X N, the

relative glomerular blood volume (RGBV) was calculated as described by Palkovits and Zolani[15] where GV is the glomerular volume and N is the average glomerular number. Sperber[16] elucidated the following formula to calculate the relative medullary thickness as RMT = medullary thickness X 10 ÷ (kidney volume)1/3, the kidney volume is the product of the multiplying length, width, and height of the kidney.

Malondialdehyde (MDA) and reduced glutathione (GSH) analyses

Lipid peroxidation was estimated by measuring the thiobarbituric acid reactive substances colorimetrically. This method is based on the malondialdehyde (MDA) determination as an end product of lipid peroxidation, which can react with thiobarbituric acid to yield a pink-colored mixture measured at 532 nm[17]. We estimated the concentration of reduced glutathione (GSH) according to the method of Ellman[18]. This method is based on the reduction of 5, 5'-Dithiobis-2-nitrobenzoic acid with GSH to yield a yellow color, which is measured at 412 nm spectrophotometrically[18].

Catalase (CAT) and superoxide dismutase (SOD) assays

According to the method of Xu *et al*.[19], the catalase activity (CAT) was determined. We have estimated superoxide dismutase (SOD) after the method of Kakkar *et al*.[20]. This method is based on inhibiting the composition of NADH-phenazine methosulphate nitroblue tetrazolium formazan. The formed color at the end of the reaction can be extracted into butanol and measured at 560 nm spectrophotometrically[20].

Immunohistochemical investigation

Kidney sections were dewaxed and rinsed in phosphate buffer saline and incubated with normal serum. These sections were incubated for 2 hours at 4°C with 100 µl of monoclonal rodent bcl2 and p53 antibodies at a dilution of 1:1000 and 1:400, respectively. These sections were rinsed in phosphate buffer saline then in biotinylated secondary antibody for 15 min then washed with phosphate buffer and avidin-biotin for 15 min. The reaction was detected using DAB (3, 3'-Diaminobenzidine) for 3 min a brown color then rinsed with distilled water. These sections were counter-stained with hematoxylin, dehydrated in ascending grades of alcohol, cleared, and mounted. The positive cell numbers were randomly selected from three different sections in each region using X 40 objective lens and counted in 30 fields[21].

Statistical analysis

Comparison between the three studied rodents was carried out using one-way ANOVA. The P values which recorded less than 0.05 were considered to be statistically significant. All statistical analyses were performed using SPSS statistical version 20.0 software package (SPSS® Inc., USA).

RESULTS

The obtained results presented in Table 1 showed no significant difference among New Valley, Faiyum, and Eastern Desert rodents in kidney weight to body weight parameters. Although, they were significantly different $P < 0.05$ in body weight and kidney weight parameters when comparing the studied rodents. The average body weight of *G. pyramidum* from the New Valley region was 24.09±6.03 g and the average kidney weight was 0.09±0.01 g. For *M. musculus* from the Faiyum region, the average body weight was 11.18±4.07 g and the average kidney weight was 0.07±0.02 g. The average body weight of *R. norvegicus* from the Eastern Desert region was 152.26±23.0 g and the average kidney weight was 0.58±0.11 g (Table 1).

Hematological and biochemical parameters

There was a significant increase in platelets, MCV, MCH, lymphocytes, monocytes, and neutrophils of *G. pyramidum*, *M. musculus*, and *R. norvegicus* from New Valley, Faiyum, and Eastern Desert regions, respectively. The greater Egyptian gerbil, *G. pyramidum*, from the New Valley region recorded a significant decrease in platelets number, MCV, MCH, and neutrophils values and recorded a significant increase in the percentage of lymphocytes and monocytes only. The house mouse, *M. musculus*, from the Faiyum region recorded a significant increase in platelets number, MCV, and MCH values and a significant decrease in the percentage of monocytes only. The Norway rat, *R. norvegicus*, from the Eastern Desert region recorded a significant increase in the percentage of neutrophils and a significant decrease in the percentage of lymphocytes only (Table 2).

Serum albumin was the only parameter recorded an insignificant difference among the studied rodents. Except for this, all tested biochemical parameters were significantly different $P < 0.05$. *G. pyramidum* from the New Valley region recorded a significant decrease in the values of urea, uric acid, total protein, potassium, calcium–total and ionized, phosphorous and magnesium. *G. pyramidum* recorded six significantly increased biochemical parameters which are ALT, AST, cholesterol, triglycerides, sodium, and chloride (Table 3).

Also, *M. musculus* from the Faiyum region recorded six significantly increased biochemical parameters which are urea, creatinine, uric acid, alkaline phosphatase, calcium–ionized, and phosphorous. *M. musculus* recorded a significant decrease in ALT, cholesterol, triglycerides, sodium, and chloride values (Table 3).

Three biochemical parameters were recorded as a significant decrease for *R. norvegicus* from the Eastern Desert region which are creatinine, AST, and alkaline phosphatase. Whereas, four biochemical parameters were recorded as a significant increase for *R. norvegicus* which are total protein, potassium, calcium, and magnesium (Table 3).

Histological investigations

(Figure 2) shows the normal histological kidney structure of the greater Egyptian gerbil, *G. pyramidum*, from the New Valley region at the cortex, outer and inner medulla. (Figure 3) shows the normal histological kidney structure of the house mouse, *M. musculus*, from the Faiyum region at the cortex, outer and inner medulla. (Figure 4) shows the normal histological kidney structure of the Norway rat, *R. norvegicus*, from the Eastern Desert region at the cortex, outer and inner medulla.

Data in (Table 4) show that there was a significant difference $P < 0.05$ among rodents from the New Valley, Faiyum, and Eastern Desert regions in all average values of the kidney parameters measured in this study except two parameters only; RMT and juxtamedullary glomerular diameter. Thirteen kidney parameters were measured in the current study, eight of which were recorded as significantly increase $P < 0.05$ for the Norway rat, *R. norvegicus*, from the Eastern Desert region. Three kidney parameters only were recorded as significantly increase $P < 0.05$ for the greater Egyptian gerbil, *G. pyramidum*, from the New Valley region. The house mouse, *M. musculus*, from the Faiyum region recorded a significant decrease for eleven parameters measured in the current study.

The highest value of the kidney volume of 670.50±60.06 mm3 was recorded for *R. norvegicus* from the Eastern Desert region and the lowest value was 100.0±43.40 mm3 for *M. musculus* from the Faiyum region. The highest value of the cortical thickness of 1.32±0.16 mm was recorded for Eastern Desert rodents and the lowest one was 0.68±0.04 mm for Faiyum mice. The whole medullary thickness was 1.88 mm for Eastern Desert rodents, 1.56 mm for New Valley gerbils, and 1.00 mm for Faiyum mice. The highest relative medullary thickness value 2.83±0.45 was recorded for New Valley gerbils and the lowest RMT 2.15±0.50 for Faiyum mice. The maximum superficial glomerular number of 168.0±5.53 was recorded for New Valley gerbils and the lowest one was 96.25±3.33 for Faiyum mice. The maximum superficial glomerular diameter, volume, and RGBV were 66.80±9.16 µm, 16.68±5.33 (x 10^4 μ^3), and 20.72±6.89 (x 10^6) for New Valley gerbils, respectively. The minimum superficial glomerular diameter, volume and RGBV were 49.36±4.60 µm, 6.27±1.70 (x 10^4 μ^3) and 6.05±1.72 (x 10^6) for Faiyum mice, respectively. The maximum values of juxtamedullary glomerular number 116.25±6.94 and juxtamedullary RGBV 33.25±8.17 (x 10^6) were recorded for New Valley gerbils. The minimum values of juxtamedullary glomerular number 54.38±6.14 and juxtamedullary RGBV 10.92±3.72 (x 10^6) were recorded for Faiyum mice. The maximum juxtamedullary glomerular diameter of 85.92±11.27 µm and volume 33.92±13.60 (x 10^4 μ^3) were recorded for New Valley gerbils. The minimum juxtamedullary glomerular diameter of 71.44±8.27 µm and volume 20.34±7.26 (x 10^4 μ^3) were recorded for Faiyum mice.

Oxidative stress parameters

The obtained results of all oxidative stress parameters, GSH, SOD, CAT, and MDA, showed significant difference $P < 0.05$ among the studied rodents. The highest values of GSH, SOD, and CAT, and the lowest values of MDA were recorded for *G. pyramidum* from the New Valley region. The minimum values of GSH, SOD, and CAT, and the maximum values of MDA were recorded for *M. musculus* from the Faiyum region. The New Valley gerbils recorded 8.8±2.7 mmol/g fresh weight for GSH, 121.3±1.4 Ul/g fresh weight for SOD, 80.9±4.1 Ul/g fresh weight for CAT, and 13.0±2.3 mmol/g fresh weight for MDA. Faiyum mice recorded 1.2±0.4 mmol/g fresh weight for GSH, 59.7±1.4 Ul/g fresh weight for SOD, 48.1±2.6 Ul/g fresh weight for CAT, and 37.6±2.0 mmol/g fresh weight for MDA. Eastern Desert rodents recorded 5.3±0.7 mmol/g fresh weight for GSH, 91.9±1.4 Ul/g fresh weight for SOD, 65.2±4.1 Ul/g fresh weight for CAT, and 16.9±3.6 mmol/g fresh weight for MDA (Figure 5).

Immunohistochemical observations

Investigation of the *G. pyramidum* kidney sections at the cortex and medulla from the New Valley region showed weak expression of the bcl2 anti-apoptotic protein (25% of the cells, stained). While positive strong immunohistochemical expression of bcl2 for both cortex and medulla (61-100% of the cells, stained) as seen by the brown color was observed for *M. musculus* from the Faiyum region. Also, weak expression of the bcl2 anti-apoptotic protein (25% of the cells, stained) was observed for *R. norvegicus* kidney sections at the cortex and medulla from the Eastern Desert region (Figuer 6).

The examination of the *G. pyramidum* kidney sections at the cortex and medulla from the New Valley region showed weak expression of the pro-apoptotic protein p53 for both cortex and medulla (25% of the cells, stained). While positive moderate for cortex (26-60% of the cells, stained) and strong immunohistochemical expression of p53 for medulla (61-100% of the cells, stained) as seen by the brown color were observed for *M. musculus* from Faiyum region. Also, weak expression of the p53 (25% of the cells, stained) was observed for *R. norvegicus* kidney sections at the cortex and medulla from the Eastern Desert region (Figure 7)

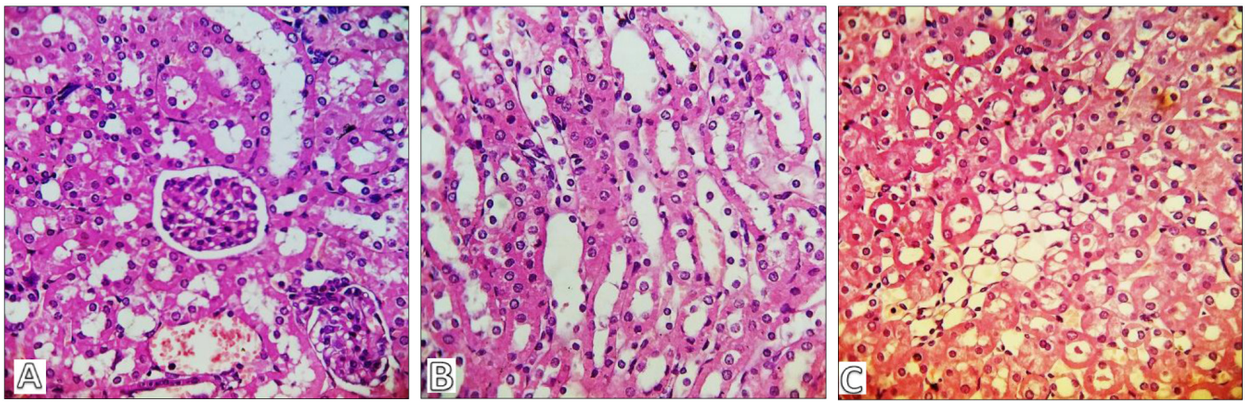

Fig. 2: Photomicrograph of renal cortex (A), outer medulla (B) and Inner Medulla (C) of *G. pyramidum* from New Valley. H & E stain (X 400).

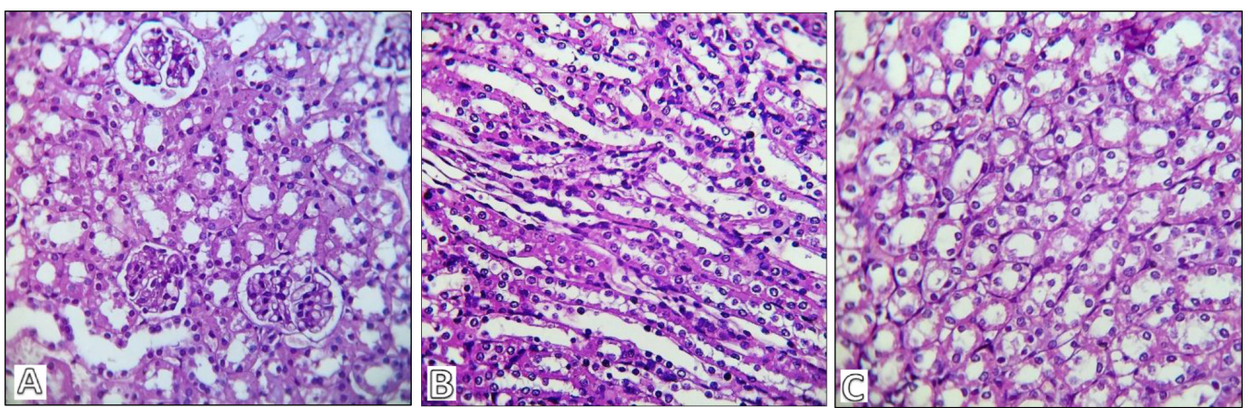

Fig. 3: Photomicrograph of renal cortex (A), outer medulla (B) and Inner Medulla (C) of *M. musculus* from Faiyum. H & E stain (X 400).

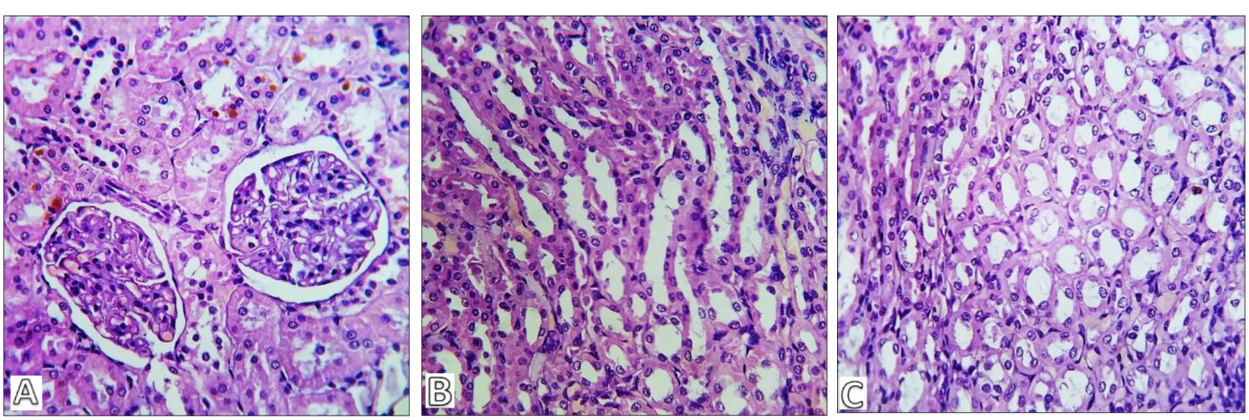

Fig. 4: Photomicrograph of renal cortex (A), outer medulla (B) and Inner Medulla (C) of *R. norvegicus* from Eastern Desert. H & E stain (X 400).

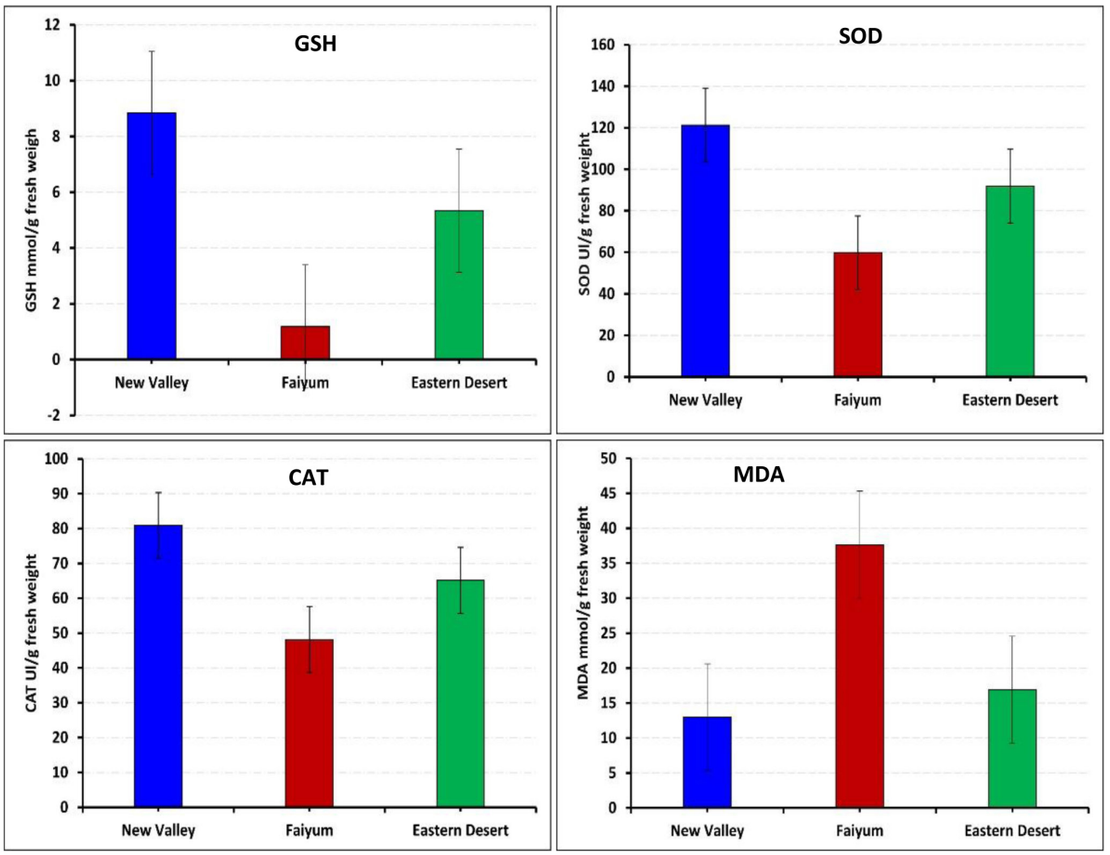

Fig. 5: Kidney antioxidant enzymes in the studied rodents. The values are represented as mean±SE, significantly different $P < 0.05$. GSH: reduced glutathione, SOD: superoxide dismutase, CAT: catalase and MDA: malondialdehyde.

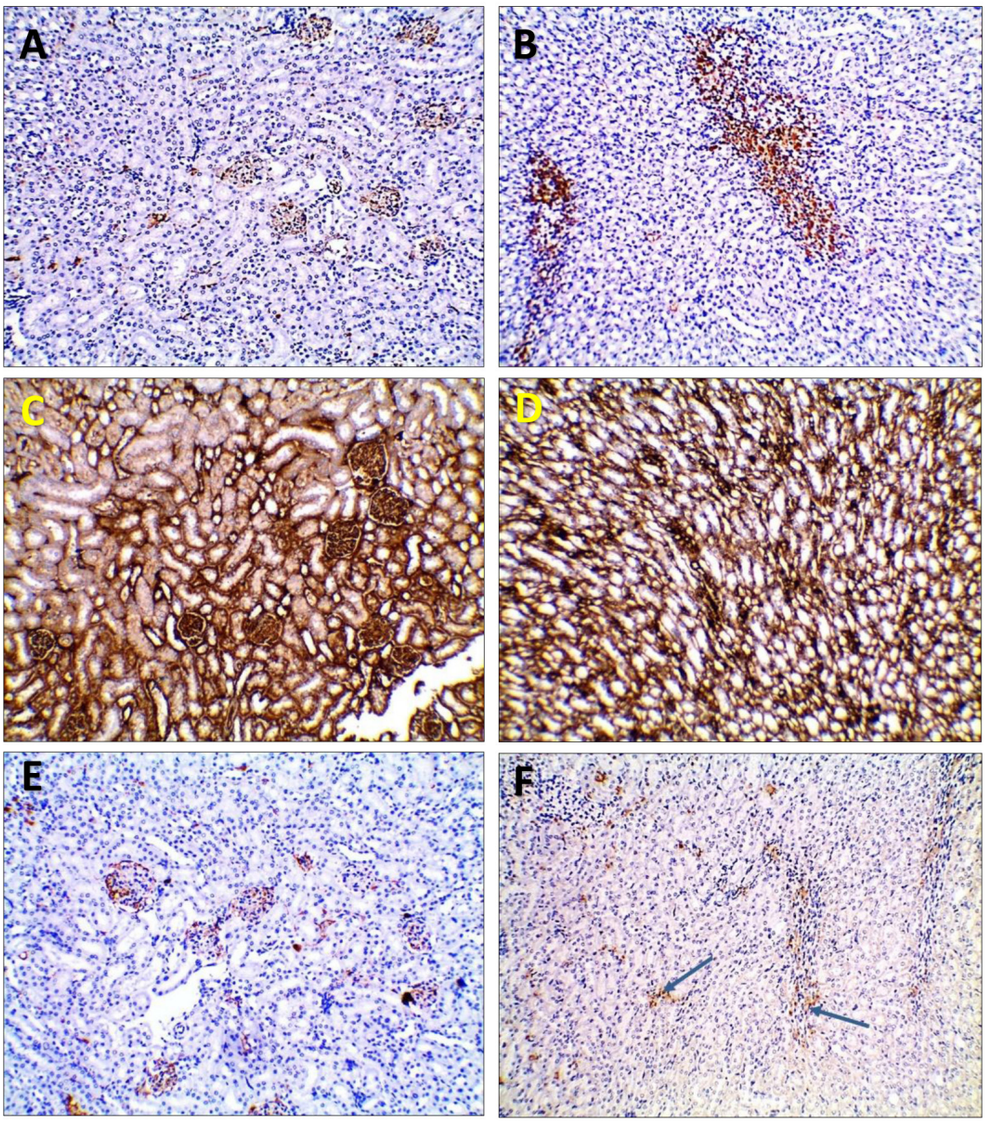

Fig. 6: Light photomicrographs of the studied rodents' kidney sections (cortex at left and medulla at right) immunostained with anti-apoptotic protein bcl2 (magnification 200 X). A and B (the New Valley gerbils) showing weak bcl2 expression for both cortex and medulla (25% of the cells, stained). C and D (the Faiyum mice) showing positive strong immunohistochemical expression of bcl2 for both cortex and medulla as indicated by the brown color (61-100% of the cells, stained). E and F (the Eastern Desert rodents) showing weak bcl2 expression for both cortex and medulla (25% of the cells, stained) (blue arrows).

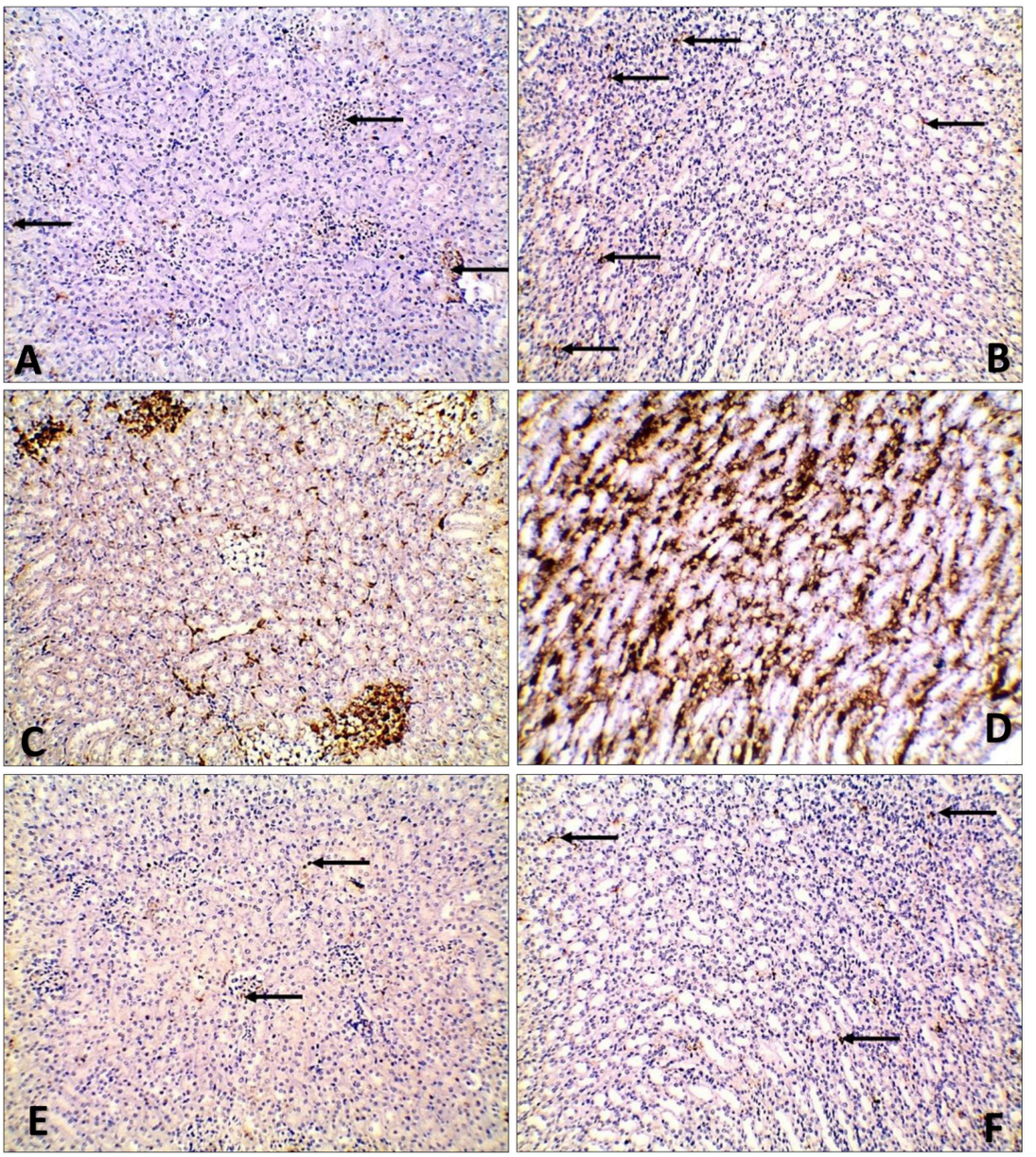

Fig. 7: Light photomicrographs of the studied rodents' kidney sections (cortex at left and medulla at right) immunostained with pro-apoptotic protein p53 (magnification 200 X). A and B (the New Valley gerbils) showing weak p53 expression for both cortex and medulla (25% of the cells, stained) (black arrows). C and D (the Faiyum mice) showing positive moderate for cortex (left) and strong immunohistochemical expression of p53 for medulla (right) as indicated by the brown color (61-100% of the cells, stained). E and F (the Eastern Desert rodents) showing weak p53 expression for both cortex and medulla (25% of the cells, stained) (black arrows).

Table 1: Body and kidney weights of the studied rodents collected from New Valley, Faiyum and Eastern Desert regions

Items	New Valley *G. pyramidum*	Faiyum *M. musculus*	Eastern Desert *R. norvegicus*
Body wt (g)	24.09* ± 6.03	11.18* ± 4.07	152.26* ± 23.00
Kidney wt (g)	0.09* ± 0.01	0.07* ± 0.02	0.58* ± 0.11
Kidney wt /Body wt X 10^{-3}	3.92 ± 0.72	5.94 ± 1.28	3.89 ± 0.96

Displayed data as mean±SD, * significant at $p < 0.05$

Table 2: Hematological parameters of the studied rodents from New Valley, Faiyum and Eastern Desert regions

Items	New Valley *G. pyramidum*	Faiyum *M. musculus*	Eastern Desert *R. norvegicus*	The reference values
RBCs X 10^6/µl	6.33 ± 0.92	6.23 ± 0.48	6.88 ± 0.27	4.7-6.1 X 10^6/µl
WBCs X 10^3/µl	8.13 ± 2.41	7.86 ± 1.29	7.33 ± 0.30	4.5-11.0 X 10^3/µl
Platelets X 10^3/mm^3	322.0* ± 88.65	563.75* ± 28.75	442.66* ± 22.07	150-400 X 10^3/mm^3
Hb (g/dl)	13.58 ± 2.12	11.85 ± 1.78	12.74 ± 0.66	12- 6 g/dl
Hct (%)	43.43 ± 6.78	42.99 ± 3.83	44.74 ± 3.09	40.7-50.3 %
MCV (femtoliters)	68.88* ± 6.17	80.66* ± 1.93	78.55* ± 1.51	80-95 femtoliters
MCH (picograms)	21.43* ± 1.85	31.75* ± 1.91	20.53* ± 0.56	27-31 picograms
MCHC (g/dl)	31.26 ± 0.05	32.0 ± 1.60	27.41 ± 1.58	32 to 36 g/dl
Lymphocytes (%)	67.75* ± 9.81	61.88* ± 1.36	45.78* ± 14.83	20-40%
Monocytes (%)	3.63* ± 0.52	1.50* ± 0.53	3.24* ± 0.35	2-8%
Neutrophils (%)	32.25* ± 9.81	37.88* ± 1.96	51.48* ± 12.88	55-70%
Eosinophils (%)	1.75 ± 0.71	2.00 ± 0.93	1.25 ± 0.71	1-4%

Displayed data as mean±SD, * significant at $p < 0.05$

Table 3: Biochemical parameters of the studied rodents from New Valley, Faiyum and Eastern Desert regions

Items	New Valley *G. pyramidum*	Faiyum *M. musculus*	Eastern Desert *R. norvegicus*	The reference values
Kidney functions				
Urea (mg/dl)	54.35* ± 7.97	102.88* ± 13.13	60.23* ± 11.49	15-45 mg/dl
Creatinine (mg/dl)	0.80* ± 0.30	1.80* ± 0.52	0.67* ± 0.05	0.7-1.4 mg/dl
Uric acid (mg/dl)	4.43* ± 0.85	8.48* ± 1.08	4.80* ± 1.06	3.6-7.7 mg/dl
Liver parameters				
ALT (U/l)	73.25* ± 17.04	18.25* ± 2.19	23.34* ± 1.91	Up to 40 U/l
AST (U/l)	62.25* ± 18.27	33.63* ± 1.60	32.13* ± 4.42	Up to 38 U/l
T. Protein (g/dl)	5.58* ± 0.24	8.05* ± 0.19	8.81* ± 0.36	6.6-8.3 g/dl
Albumin (g/dl)	3.20 ± 0.21	4.67 ± 0.06	4.63 ± 0.35	3.5-5.0 g/dl
Cholesterol (mg/dl)	225.13* ± 72.12	112.0* ± 6.09	184.31* ± 22.64	< 200 mg/dl
Triglycerides (mg/dl)	276.0* ± 50.63	160.13* ± 3.27	263.72* ± 17.24	40-160 mg/dl
Alkaline Phosphatase (U/l)	153.75* ± 44.88	272.63* ± 3.38	104.19* ± 18.68	98-279 U/l
Serum electrolytes				
Potassium (K) (mmol/l)	5.35* ± 0.15	6.69* ± 0.18	7.71* ± 0.20	3.5-5.1 mmol/l
Sodium (Na) (mmol/l)	163.88* ± 3.52	141.50* ± 2.07	157.03* ± 2.22	136-146 mmol/l
Chloride (Cl) (mmol/l)	123.50* ± 4.24	107.13* ± 1.64	107.50* ± 1.85	95-115 mmol/l
Calcium – total (mg/dl)	2.54* ± 0.15	9.20* ± 0.06	13.22* ± 0.15	8.5-10.5 mg /dl
Calcium – ionized (mg/dl)	1.33* ± 0.04	5.31* ± 0.30	4.35* ± 0.16	4.8-5.6 mg/dl
phosphorous (PO4) (mg/dl)	7.27* ± 0.14	19.23* ± 0.25	13.40* ± 0.19	2.5-5.0 mg/dl
Magnesium (Mg) (mg/dl)	1.73* ± 0.14	2.61* ± 0.13	2.81* ± 0.11	1.6-2.5 mg/dl

Displayed data as mean±SD, * significant at $p < 0.05$

Table 4: Kidney measurement parameters of the studied rodents from the New Valley, Faiyum and Eastern Desert regions

Items	New Valley *G. pyramidum*	Faiyum *M. musculus*	Eastern Desert *R. norvegicus*
Kidney Volume (mm^3)	172.13* ± 31.0	100.0* ± 43.40	670.50* ± 60.06
Cortical Thickness (mm)	0.85* ± 0.10	0.68* ± 0.04	1.32* ± 0.16
Outer Medullary Thickness (mm)	0.70* ± 0.13	0.39* ± 0.04	0.84* ± 0.05
Inner Medullary Thickness (mm)	0.86* ± 0.08	0.61* ± 0.11	1.04* ± 0.11
Relative Medullary Thickness (RMT)	2.83 ± 0.45	2.15 ± 0.50	2.26 ± 0.16
Superficial Glomerular Number	168.0* ± 5.53	96.25* ± 3.33	123.88* ± 11.41
Superficial Glomerular Diameter (µm)	50.35* ± 6.06	49.36* ± 4.60	66.80* ± 9.16
Superficial Glomerular Volume x 10^4 (μ^3)	7.07* ± 2.31	6.27* ± 1.70	16.68* ± 5.33
Superficial RGBV x 10^6	11.90* ± 3.93	6.05* ± 1.72	20.72* ± 6.89
Juxtamedullary Glomerular Number	116.25* ± 6.94	54.38* ± 6.14	91.38* ± 4.37
Juxtamedullary Glomerular Diameter (µm)	82.13 ± 7.29	71.44 ± 8.27	85.92 ± 11.27
Juxtamedullary Glomerular Volume x 10^4 (μ^3)	28.69* ± 7.39	20.34* ± 7.26	33.92* ± 13.60
Juxtamedullary RGBV x 10^6	33.25* ± 8.17	10.92* ± 3.72	30.96* ± 12.33

Displayed data as mean±SD, * significant at $p < 0.05$

DISCUSSION

The studied rodents

In Egypt, the greater Egyptian gerbil, *G. pyramidum*, is known as Demsi, the house mouse, *M. musculus*, is known as Fa'r Al-manzil and the Norway rat, *Rattus norvegicus*, is known as Fa'r Nurwigi. Data in Table 1 demonstrate the average body weight of *G. pyramidum*, *M. musculus*, and *R. norvegicus* from New Valley, Faiyum, and Eastern Desert regions, respectively. Osborn and Helmy[22] mentioned that the average body length of *G. pyramidum* ranges from 10.7 to 13.1 cm; tail length from 15.0 to18.0 cm and body weight from 37.2 to 67.2 g. The average body length of *M. musculus* from 7.8 to 9.1 cm; tail length from 6.6 to 8.5 cm and body weight from 9.4 to 20.1 g. The average body length of *R. norvegicus* ranges from 16.9 to 25.4 cm; tail length from 14.5 to 23.4 cm and body weight from 208.3 to 360 g. Hoath[23] documented that the average body length of *G. pyramidum* ranges from 23.0 to 39.3 cm; tail length from 12.8 to 18.0 cm and body weight from 14 to 18 g. The average body length of *M. musculus* is from 10.8 to 20.0 cm; tail length from 5.3 to 9.7 cm and body weight from 9.4 to 20.9 g. The average body length of *R. norvegicus* ranges from 34.1 to 48.8 cm; tail length from 14.5 to 23.4 cm and body weight from 208.3 to 360 g. The results of the body weight of *G. pyramidum* (24.09 g) and *R. norvegicus* (152.26 g) presented in the current study disagreed with the findings of Osborn and Helmy[22] and Hoath[23]. While the results of the body weight of *M. musculus* (11.18 g) agreed with the findings of Osborn and Helmy[22] and Hoath[23].

Hematological parameters

Table 2 shows that there were significant differences among the studied rodents. Whereas, *G. pyramidum* recorded a significant decrease in platelets number, MCV, MCH, and neutrophils values and recorded a significant increase in the percentage of lymphocytes and monocytes only. *M. musculus* from the Faiyum region recorded a significant increase in platelets number, MCV, and MCH values and a significant decrease in the percentage of monocytes only. *R. norvegicus* from the Eastern Desert region recorded a significant increase in the percentage of neutrophils and a significant decrease in the percentage of lymphocytes only.

The normal total count of WBCs is between 2 to 10 × $10^3/mm^3$[24]. Our results agreed with Silva-Santana *et al*.[24]. The present results were lower than the normal platelets count in rodents which is from 900 to 1600 × $10^3/mm^3$ as reported by Wendland *et al*.[25].

Shahsavani *et al*.[26] documented that to evaluate the erythrogram; RBCs, Hct, Hb, MCV, MCH, and MCHC are the common hematological parameters to be used. Hb is an extremely important parameter in reduction and transformation of oxygen for hydroxylation processes. Weiss and Wardrop[27] believed that in rodents, Hb can be measured as the total volume in the RBCs and has a value from 10 to 17 g/dl. The present findings were in agreement with Weiss and Wardrop[27]. The relationship between the size of the erythrocytes and the concentration of Hb is important for diagnoses of the different types and degrees of anemia[28]. MCV is used to determine the degree of anisocytosis and the differentiation of anemia. MCH may ranging from 13 and 17 pg in rodents and can be determined from the relationship between the mean concentration of Hb inside RBC and the number of RBCs[28]. Our results were slightly increased than that mentioned by Ribeiro-Alves and Gordan[28]. The values of MCHC measure Hb concentration in RBC and range, in rodents, from 30 to 38 g/dl[27]. The current findings were in agreement with Weiss and Wardrop[27] but MCHC values for *R. norvegicus* were slightly less than that mentioned values. Different habitats and disorders may either increase or decrease these hematological parameters. The toxic stress induced by various contaminants, can be evaluated by the commonly hematological parameters such as RBCs, WBCs, Hb, Hct, MCV, MCH and MCHC. In several animals, changes to these parameters are regarded as an anaemic state.

Biochemical parameters

Measuring of biochemical parameters give an important overview of nutritional balance and clinical status of the organs and tissues, as well as evidence of many diseases[29]. The determination of kidney function is dependant on measuring the values of urea and creatinine in blood[30]. Urea is the main nitrogenous substance that is formed from the degradation of the protein. About 90% of urea is excreted by the kidneys whereas 40% to 70% returns to plasma[30].

Branco *et al*.[31] reported the blood urea value 53.00±1.90 mg/dl for rodents. This value was lower than that recorded in the current study. Riken[32] reported that rodents have an average serum creatinine of 0.28 mg/dl which formed as a result of creatine catabolism. Creatinine is present in the liver and kidneys and all different types of muscles and is mainly excreted by glomerular filtration in the kidneys[33]. Uric acid is formed in the liver as a result of purine catabolism[34]. Almeida *et al*.[35] documented lower uric acid levels 1.54±0.68 mg/dl for mice than that obtained in this study. Hyperuricemia is known as high levels of uric acid which can result from the activity of enzymes and renal excretion deficiency, urate crystals deposition, and may induce arterial hypertension[34].

Abundantly, ALT enzyme is found in the liver, in moderate levels in the kidneys, and in small parts of the skeletal muscles and heart[36]. AST is found in various organs and tissues, especially in the mitochondria and cytoplasm of the heart, kidneys, liver, brain, erythrocytes, and some muscles. In rodents, Rusyn and Threadgill[37] reported ALT and AST concentrations are 41 U/l and 152 U/l, respectively.

In rodents, Yuan and Korstanje[38] stated alkaline phosphatase and total protein levels are 86 U/l and 5.22 g/dl, respectively. Barbosa *et al*.[39] reported values of 29.72 U/l for alkaline phosphatase and 2.32 g/dl for total protein. The current study results showed higher levels of alkaline phosphatase and total protein than that reported by Yuan and Korstanje[38] and Barbosa *et al*.[39]. Alkaline phosphatase is found in the epithelial cells of the bile duct, osteoblasts of bones, kidneys, intestines, and placenta[36]. The determination of total protein in the blood is an important test of metabolism homeostasis. Proteins are found in all cell structures, being essential for their components[40].

Alternations of the ALT, AST, alkaline phosphatase, and total protein levels in the blood can be considered as a mirror of some diseases caused by various organs. Whereas the rapid elevation of ALT enzyme refers to hepatic diseases[36].

Albumin comprises 50% of the total protein in the blood. Globulin is obtained from the difference between total protein and albumin. Harris[41] stated albumin and globulin are important in osmotic pressure maintenance and are usually estimated in correlation with total protein. A decrease in total protein levels in the blood may be caused by the blood reduction of both albumin and globulin. Our results of serum albumin show no significant difference between the studied rodents.

Junghanns[42] reported cholesterol and triglycerides parameters can be the consequence of the diet or produced by the liver. Triglycerides can be produced from the intestinal mucosa cells and can also be produced from the liver[43].

Blood electrolytes may regulate most functions of the animal body such as acid-base balance and water regulation. In our results the blood Na, K, and Ca (total) showed a significant difference between the studied rodents. Sodium (Na) is one of the most important blood electrolytes in the animal body. The normal values accepted for serum Na is 134 to 145 mmol/l[44]. Our Na results were higher than the standard values for *G. pyramidum* and *R. norvegicus* but the Na values were in the normal range for *M. musculus*. The normal values accepted for serum K are 3.5 to 5.0 mmol/l[44]. Our K results were higher than the standard values for *G. pyramidum*, *M. musculus*, and *R. norvegicus*. Calcium (Ca) helps in nerve impulse control, the formation of bones, and muscle contraction. The normal values accepted for serum Ca ranges from 2.2 to 2.5 mmol/l. Raising blood K above its normal values stimulates the aldosterone kaliuretic action[44]. Our Ca results were higher than the standard values for *M. musculus* and *R. norvegicus* but the Ca values were in the normal range for *G. pyramidum*.

The normal values of serum chloride are 97-105 mmol/l. PO4 plays a vital role in most biochemical reactions related to energy production. The international normal levels of PO4 is ranging from 2.5-4.5 mg/dl[45]. When blood PO4 increases above 4.5 mg/dl, hyperphosphatemia may occurs. The current study results of Cl and Po4 levels revealed that there was an increase in their values above the standard values for all studied rodents.

Magnesium (Mg) plays an essential role in many cellular reactions[46]. The standard levels of Mg are 1.5-2.5 mmol/l. Hypermagnesemia occurs when the level of blood magnesium is more than 2.5 mmol/l and hypomagnesemia occurs when serum magnesium is lower than 1.5 mmol/l[47]. Our Mg results were higher than the standard values for *M. musculus* and *R. norvegicus* but the Mg values were in the normal range for *G. pyramidum*.

Histological investigations

The present study showed a significant difference among most of the kidney parameters measured for the studied rodents in the values of kidney volume, cortical and medullary thickness, superficial and juxtamedullary glomerular numbers, volumes, and RGBV. In the kidney of the studied rodents, there was a great variation in size between their glomeruli. Our results show that the average juxtamedullary glomeruli diameter was greater 1.63 times than the superficial glomeruli of *G. pyramidum*. The average

juxtamedullary glomeruli diameter was greater 1.45 times than the superficial glomeruli of *M. musculus*. The average juxtamedullary glomeruli diameter were greater 1.29 times than the superficial glomeruli of *R. norvegicus*. Ghalwash *et al*.[48] comparing the desert-dwelling G. gerbillus inhabiting two different habitats. They reported that the average diameter of juxtamedullary glomeruli was greater 1.40 times than superficial glomeruli in gerbils captured from the Faiyum region and was greater 1.21 times in gerbils collected from the Western Mediterranean Coastal Desert region from Egypt. The current findings agreed with those reported by Ghalwash *et al*.[48]. It is becoming clear that the kidney's capacity to concentrate urine is influenced by several variables other than medullary thickness. Bankir and de Rouffignac[49] reported that these structural and physiological characteristics have been highlighted by in a wide variety of ways. They point out that, in addition to the loop of Henle's length, the development and maintenance of the medullary concentration gradient may also be influenced by the presence of fornices in the renal pelvis, the degree of heterogeneity in nephron dimensions, the structural organisation of the collecting ducts and renal vasculature, the development of the outer and inner medulla, and the degree of urea recycling by the kidney.

Our study showed that the average juxtamedullary glomeruli volume was greater 4.06 times than superficial glomeruli of *G. pyramidum*. The average juxtamedullary glomeruli volume was greater 3.24 times than the superficial glomeruli of *M. musculus*. The average juxtamedullary glomeruli volume was greater 2.03 times than the superficial glomeruli of *R. norvegicus*. Ghalwash *et al*.[48] reported that the average volume of juxtamedullary glomeruli was greater 2.76 times than superficial glomeruli in gerbils collected from the Faiyum region and greater 1.78 times in gerbils collected from the Western Mediterranean Coastal Desert region from Egypt. The current findings of *R. norvegicus* agreed with those reported by Ghalwash *et al*.[48] but greater in *G. pyramidum* and *M. musculus* than reported by Ghalwash *et al*.[48]. Our findings disagreed with Munkacsi and Palkovits[50] who reported that the juxtamedullary glomeruli were 0.28 larger than the cortical glomeruli in *R. norvegicus*.

The variation between the size and numbers of juxtamedullary and superficial glomeruli indicates kidney adaptation which had been first observed in the desert rodent by Munkacsi and Palkovits[50]. This variation in size and numbers indicates the glomerular filtration would be greater in the large juxtamedullary glomeruli than the small cortical glomeruli.

One of the important adaptations in desert rodents is the increase in the thickness of the kidney's inner medulla. The current results of the inner medullary thickness (IMT) of the kidney were 0.86 mm, 0.61 mm, and 1.04 mm for *G. pyramidum*, *M. musculus*, and *R. norvegicus*, respectively. It is wrong to compare the absolute measurement of the inner medullary thickness of the kidney but to make a good comparison, we divide this absolute measurement by the kidney volume (IMT/kidney volume) for each studied rodent separately. So the IMT of *M. musculus* is greater when compared with the other studied rodents. Also, we observed no significant difference among the studied rodents in relative medullary thickness (RMT). Ghalwash *et al*.[48] documented that the IMT of the rodents collected from Faiyum Depression were 0.63 mm and 0.68 mm for those collected from WMCD in Egypt. These authors stated RMT was increased significantly in WMCD rodents when compared with Faiyum Depression rodents. The current study findings disagreed with Ghalwash *et al*.[48].

The obtained results of all oxidative stress parameters showed a significant difference among the studied rodents. The highest values of GSH, SOD, and CAT, and the lowest values of MDA were recorded for *G. pyramidum* from the New Valley region. The minimum values of GSH, SOD, and CAT, and the maximum values of MDA were recorded for *M. musculus* from the Faiyum region. Antioxidant enzymes reduce reactive oxygen species and prevent cell membranes from damage[51]. Catalase is an enzyme that degrades H_2O_2 into water and oxygen, thus rendering it harmless[52]. After oxidative stress, the formed H_2O_2 stimulates an increase in catalase level. Glutathione is the most fundamental antioxidant molecule in the cell. It is produced in a reaction catalyzed by glutathione peroxidase, GSH is the reduced form of glutathione and reacts with H_2O_2 or lipid peroxides to neutralize these molecules[53]. It is found in tissues both in their reduced state and in an oxidized state. A decrease or an increase in GSH levels in the tissues is considered an indicator of oxidative stress[54]. Superoxide dismutase converts superoxide radicals into oxygen and H_2O_2. It plays an essential role in the antioxidant defense process. SOD deficiency results in the accumulation of reactive oxygen species in tissues[55]. Reactive oxygen species form due to oxidative stress-induced lipid peroxidation. Malondialdehyde is a free aldehyde and a toxic by-product of lipid peroxidation. MDA is considered an indicator of lipid peroxidation and acts as oxidative stress agent[56]. This interprets why kidney GSH levels decreased in the mice collected from the Faiyum region when compared with those from the New valley and Eastern desert regions in the current study. We also observed a decrease in SOD and CAT levels in the Faiyum region mice. This is maybe because SOD is a metalloenzyme and could induce dismutation of endogenous cytotoxic superoxide radicals to produce H_2O_2. This product of H_2O_2 is harmful to both fatty acids and proteins which are responsible for cell membrane forming. So, the catalase enzyme is responsible for disposing of hydrogen peroxide produced by the superoxide dismutase. Ghalwash *et al*.[48] reported the same results on gerbils collected from Faiyum Depression and the Western Mediterranean Coastal Desert in Egypt.

In our study, the investigation of the *G. pyramidum* kidney sections at the cortex and medulla from the New Valley region showed weak expression of the bcl2 anti-apoptotic protein and showed weak expression of the pro-apoptotic protein p53. While positive strong

immunohistochemical expression of bcl2 for both cortex and medulla was observed for *M. musculus* from the Faiyum region. Also, moderate positive reaction for cortex and strong immunohistochemical expression of p53 for medulla were observed for *M. musculus* from the Faiyum region. A weak expression of the bcl2 anti-apoptotic protein and weak expression of the p53 was observed for *R. norvegicus* kidney sections at the cortex and medulla from the Eastern Desert region. The explanation of these results may be due to the unpolluted desert environment in which the *G. pyramidum* lives in the New Valley region. While the Faiyum region is considered an area with several agricultural fields and urbanization which may cause more environmental pollution. It is known that bcl2 expression increases according to the differentiation rate of lesions. Whereas, high bcl2 immunoreactivity rates may be found in well-differentiated lesions, which would essentially have better prediction[57]. The current study agreed with Ghalwash *et al*.[48] who studied bcl2 and p53 on the kidneys of two gerbil species from Egypt.

CONCLUSIONS

G. pyramidum from the New Valley region has the highest RMT, superficial glomerular number, diameter, volume, RGBV, juxtamedullary glomerular number, and RGBV. Also, it revealed the highest values of GSH, SOD, and CAT and the lowest values of MDA. Weak expression of both anti-apoptotic protein bcl2 and pro-apoptotic protein p53 were observed for these gerbils' kidney tissues. *M. musculus* from the Faiyum region has the lowest RMT, superficial glomerular number, diameter, volume, and RGBV, juxtamedullary glomerular number, diameter, volume, and RGBV. It showed the lowest values of GSH, SOD, and CAT, and the highest values of MDA. Positive strong immunoreactivity of bcl2 for both cortex and medulla and positive moderate of p53 for cortex and strong expression for medulla were observed for these mice's kidney tissues. In *R. norvegicus* from the Eastern Desert region, the largest body weight, have greater kidney volume, cortical and medullary thickness, juxtamedullary glomerular diameter, and volume. Weak expression of both anti-apoptotic protein bcl2 and pro-apoptotic protein p53 were observed for these rodents' kidney tissues.

CONFLICT OF INTERESTS

There are no conflicts of interest.

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الملخص العربى

دراسة مقارنة للدم وكيمياء الدم الحيوية وأنسجة الكلى للعضل المصري الكبير والفأر المنزلي و الجرذ النرويجي من ثلاث مناطق مختلفة في مصر

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الخلفية: تعتبر القوارض أفضل أنواع الثدييات لدراسة التصنيف والبيولوجيا (التركيب النسيجي ووظائف الأعضاء). وتُعد الجرابيع والفئران والجرذان من أنواع الثدييات المفضلة للدراسة نظرًا لصغر حجمها وقصر دورة حياتها وسرعة تكاثرها.

الهدف من العمل: نحاول إظهار البنية النسيجية ووظائف الكلى، والإجهاد التأكسدي، والتعبير الكيميائي المناعي للعضل المصري الكبير والفأر المنزلي والجرذ النرويجي التي تم جمعها من ثلاثة بيئات مختلفة.

الطرق والأدوات: تم تجميع ٢١ عينة حية متمثلة في ٧ عينات من العضل المصري الكبير من منطقة الوادي الجديد، و٨ عينات من الفأر المنزلي من منطقة الفيوم و٦ عينات من الجرذ النرويجي من منطقة الصحراء الشرقية.

النتائج: سجل العضل المصري الكبير زيادة ملحوظة في ستة قياسات كيميائية حيوية وهي إنزيمات ALT وAST والكوليسترول والدهون الثلاثية والصوديوم والكلور. كما سجل الفأر المنزلي أيضاً ستة قياسات ذات زيادة ملحوظة وهي اليوريا والكرياتينين وحمض البوليك والفوسفاتيز القلوي والكالسيوم المتأين والفوسفات. بينما سُجلت الزيادة الملحوظة لأربع قياسات فقط هي البروتين الكلي والبوتاسيوم والكالسيوم الكلي والماغنيسيوم للجرذ النرويجي. وأكدت النتائج أن قوارض منطقة الوادي الجديد، التي تعيش في منطقة صحراوية قاحلة، تمتلك كُلى أكثر كفاءة من تلك التي تعيش في مناطق الفيوم والصحراء الشرقية. وقد تم تسجيل أكبر سمك نسبي لنخاع الكُلى عند ٢,٨٣±٠,٤٥ لقوارض منطقة الوادي الجديد والأقل عند ٢,١٥±٠,٥٠ لفئران منطقة الفيوم. وأظهرت الدراسة أن متوسط قطر الكبيبات المجاورة للنخاع أكبر ١,٦٣ مرة من الكبيبات السطحية للعضل المصري الكبير، وأكبر ١,٤٥ مرة للفأر المنزلي وأكبر ١,٢٩ مرة للجرذ النرويجي. كما تم تسجيل أعلى قيم لـ GSH وSOD وCAT لكلية العضل المصري الكبير وسُجلت أدنى قيم لـ GSH وSOD وCAT لكلية الفأر المنزلي. وبينت النتائج أن قوارض منطقتي الوادي الجديد والصحراء الشرقية ذات تعبير ضعيف في الاستجابة الكيميائية المناعية لكل من bcl٢وp٥٣ في كلاً من قشرة ونخاع الكُلى. بينما سجلت فئران الفيوم تفاعلاً قوياً في الاستجابة الكيميائية المناعية لـ bcl٢ وتعبيراً متوسطاً لـ p٥٣ في قشرة الكُلى وتفاعلاً قوياً لـ p٥٣ في نخاع الكُلى.

الخلاصة: يمتلك العضل المصري الكبير من منطقة الوادي الجديد أعلى سمك نسبي لنخاع الكُلى RMT، وعدد كبيبات سطحية أكبر، وقطر وحجم كبيبات سطحية أكبر وRGBV للكبيبات السطحية أكبر، وعدد كبيبي مجاور للنخاع، و RGBV للكبيبات المجاورة للنخاع أكبر من تلك القوارض الأخرى محل الدراسة.