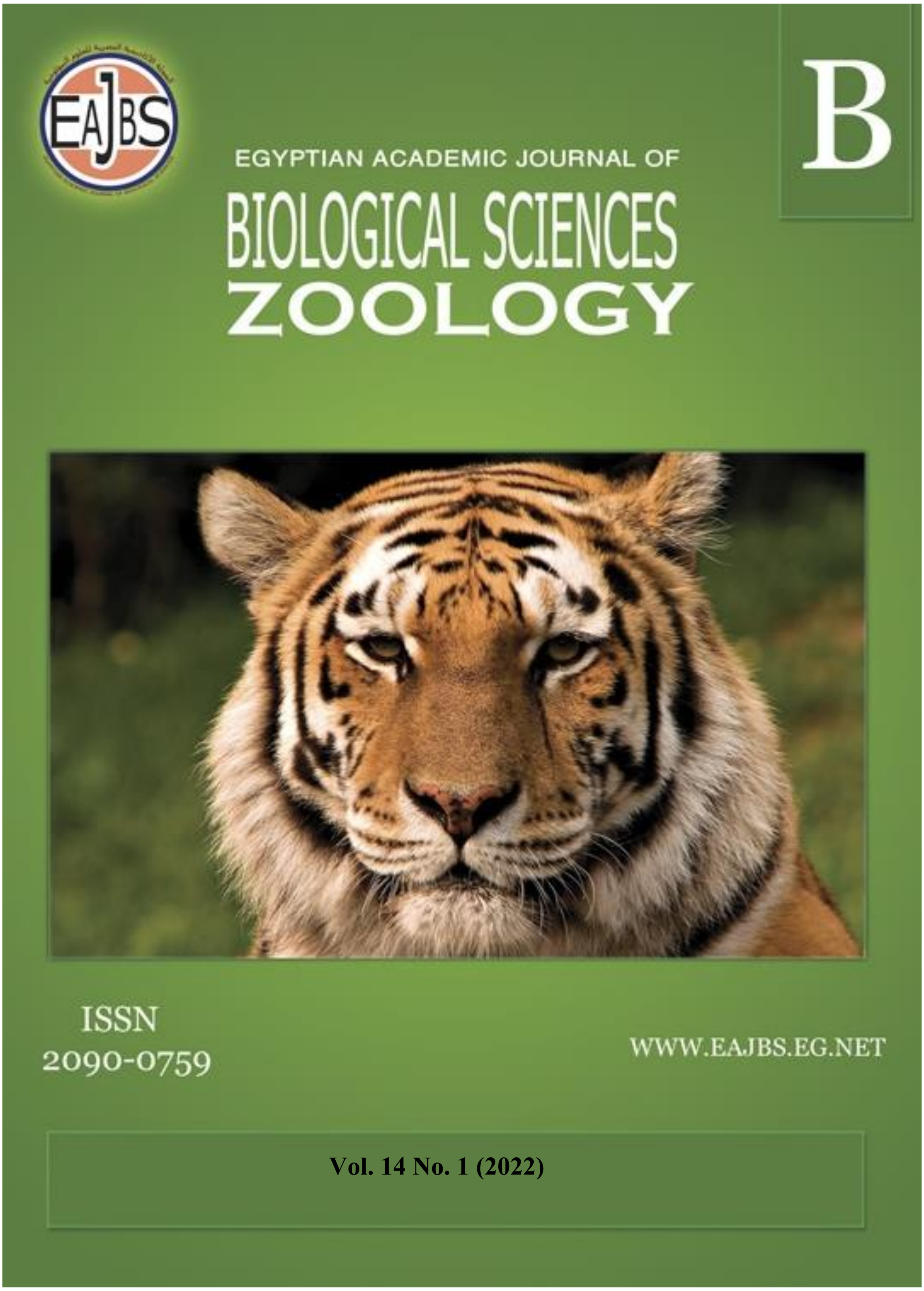

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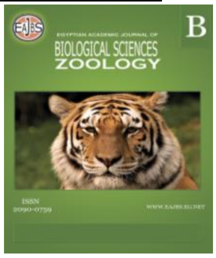

Kidney Function and Structure, Oxidative Stress and Immunohistochemical Expression of *Gerbillus gerbillus* from Two Different Habitats in Egypt

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ABSTRACT

Mammals from arid habitats have greater relative medullary thickness and maximum urine concentration as compared with those from mesic habitats. The Egyptian lesser gerbil, *Gerbillus gerbillus* (Olivier, 1801), inhabits coastal dunes of white nummulitic limestone sand in the Western Mediterranean coastal belt. In Faiyum it inhabits sandy patches in cultivated areas, palm groves and sandy areas of semi-desert supporting grasses. The present study used a total of 27 (14 from North Coast region and 13 from Faiyum region) live specimens of *G. gerbillus*. The results showed that the gerbils from North Coast have kidneys that are more efficient than those that live in Faiyum region. Although the glomerular diameter and volume in the gerbils' kidney from Faiyum region were larger than those from North Coast region unless the glomerular number and relative medullary thickness (RMT) in gerbils' kidney from North Coast region were greater than those from Faiyum region. The whole medullary thickness was 1.11 (mm) for gerbils from North Coast region and 1.05 (mm) for Faiyum region. The RMT was 2.31±0.25 for gerbils from North Coast region and 1.92±0.23 for Faiyum region. The gerbils from North Coast region showed a significant increase in the values of the kidney reduced glutathione (GSH), superoxide dismutase (SOD) and catalase (CAT) whereas the gerbils' kidney from Faiyum region showed a significant increase in the malondialdehyde (MDA) values only. The gerbils from North Coast region showed weak anti-apoptotic protein expression of the Bcl-2 and weak immunohistochemical expressions of the p53 in kidney tissues. The gerbils from Faiyum region showed strong anti-apoptotic protein expression of the Bcl-2 and showed moderate immunohistochemical expressions of the p53 in kidney tissues.

INTRODUCTION

The narrow western Mediterranean coastal belt is the wettest region of Egypt. Along this narrow coastal belt of the Mediterranean Sea, there are wells and cisterns fed by local rainfall. The most notable characteristic of the western Mediterranean coast is the expansion of ridges of soft oolitic limestone extending parallel to the shore for long distances (Ball, 1939). Commonly one line of ridges approaches the coast, while another runs parallel with it and there is sometimes a third ridge between the second and the edge of the Western Desert inland.

Salt-lagoons and marshes and in others a tract of loamy ground in some places may be found between the coastal ridge and the one next inland. The southern region of the Western Mediterranean coastal belt extends southwards to the Qattara Depression. Westward it increases gradually in level and attains a maximum elevation of about 200 m at Sallum, sloping gently northwards. It decreases gradually in level eastward until it loses its demarcation line with the coastal plain (Ayyad and Hilmy, 1974).

Closely connected with the River Nile is the Faiyum Depression about 1700 km^2 which lies a little to the west of the Nile Valley and to which it is connected by a narrow channel through the distant hills. The lower part of the depression is about 200 km^2 and is occupied by a shallow saline lake called Lake Qarun. The depression floor slopes downward to the lake in a northwesterly direction from about 23 m above sea level. It is a rich silt land irrigated by the Bahr Yusuf canal that enters it from the River Nile (Zahran and Willis, 2009).

Groundwater is the main source of supply for the Wadi El-Rayan in the south portion of which, and according to Ball (1927), there are three springs deriving their water from the sheet of subterranean water under the Western Desert. Fox (1951) documented these springs are about 600 m beneath the depression in the fissured Nubian Sandstone. Ball (1927) reported that the water of these springs is derived from remote collecting areas. All these springs have long been in use and their water is drinkable (Fakhry, 1947). The Faiyum Depression was inhabited and a part of it was cultivated in the first and second centuries AD (Zahran and Willis, 2009).

The mammalian kidneys have a prevalent role in controlling the volume and concentration of body fluids. The nephron is the functional unit of the kidney and consists of a glomerulus, proximal and distal convoluted tubules and well-developed loops of Henle. The vascular and the morphological structures of nephrons enable mammals to produce fluid that is more concentrated than their plasma. The nephrons that have long looped are characterized by an extended renal papilla (Folk, 1974; Bankir and de Rouffignac, 1985) and reflect the great length of the loop of Henle. The maximum length of the loop of Henle is directly proportional to the medullary thickness (Beuchat, 1996). Sperber (1944) is the first who showed a relationship between the renal papilla length and the availability of drinking water in the natural habitat of the animal. Specifically, mammals living in arid and semiarid habitats tended to have exceptionally long loops of Henle, as compared with mammals living in mesic habitats. Sperber (1944) suggested RMT as an index for quantifying the relative length of the longest Henle's loops. He found that mammals from arid habitats had higher values of the relative medullary thickness than those from more mesic habitats.

Interestingly, both maximum urine concentration and RMT scaled negatively with body mass (i.e., the larger-bodied animal had lower values). When considering the effect of habitat (with body mass as a covariate), mammals from arid habitats tended to have maximum urine concentration and greater RMT, as compared with those living in mesic habitats. The medullary thickness gave similar results when tested across habitats. Hence, allometric relationships for urine concentrating ability and RMT have been developed for mammals living in xeric (Calder and Braun, 1983; Beuchat, 1996) and those living in mesic environments (Beuchat 1996). The mammalian kidneys are responsible for several functions, which are excretion of waste products, electrolyte and volume regulation, acid-base balance, metabolism of low molecular weight proteins and synthesis of hormones such as erythropoietin (Madrazo-Ibarra and Vaitla, 2020). The aim of this study was to determine the kidney structure and functions, oxidative stress and immunohistochemical expression of *G. gerbillus* from North Coast and Faiyum regions in Egypt.

MATERIALS AND METHODS

Experimental Animals:

A total of 27 (14 from Dabaah, North Coast region and 13 from Kom O'shim, Faiyum region) (Fig. 1) live specimens of the lesser Egyptian gerbil, *G. gerbillus*, from both sexes. All rodent samples were identified and weighed.

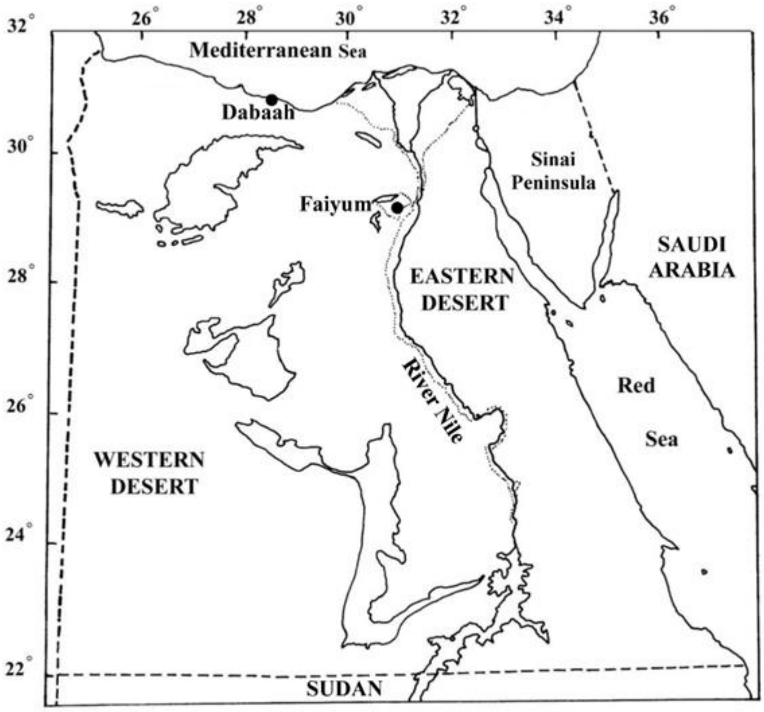

Fig. 1: Map of Egypt showing North Coast (Dabaah) and Faiyum studied regions

Blood Sampling:

The blood samples were taken in heparinized capillary tubes from the retro-orbital venous plexus and the serum was separated. A part of the blood sample was mixed with EDTA as an anticoagulant agent for measuring the hematological parameters and collecting the plasma samples. Plasma and sera were separated and stored at -20°C until analyzed. Plasma and sera were used for measuring the biochemical parameters. After dissecting animals, their kidneys were carefully removed and weighed. The kidneys of each rodent were weighed in order to calculate the ratio between kidney to body weight (KBR). KBR = weight of kidney in grams x 100/ bodyweight of animal in grams. One kidney of each rodent was embedded in paraffin for light microscopy investigation. Samples of kidneys were placed in 10% neutral buffered formalin for histological and immunohistochemical examinations. The other kidney was homogenized in 5% phosphate buffer solution pH 7.4 then centrifuged for 15 minutes at 3000 rpm at 4°C and the supernatant was carefully taken and stored at -20°C for determination of antioxidant enzymes activity.

Hematological and Biochemical Parameters:

The anticoagulated blood samples for RBCs, WBCs and platelets counts were investigated using a hemocytometer. Hemoglobin, hematocrit, MCV, MCH, MCHC and differential leucocytes were also determined. Serum urea was determined enzymatically according to the method of Tietz (1990). Serum uric acid concentration and creatinine were determined using the method of Tietz (1986). Total cholesterol, total triglycerides, potassium, sodium, chloride, phosphate (PO4), magnesium and calcium (total and ionized) levels were determined using commercial kits according to the method of Stein (1987).

Histological Examination:

One kidney was immediately removed and fixed in 10% formalin then was sectioned at 5 µm. Some sections were stained with hematoxylin and eosin (Bancroft and Cook, 1994).

Estimation of Glomerular Diameter and Volume:

The cortical, outer and inner medullary thickness of the kidney was measured using a calibrated eyepiece. The total number of glomeruli in a longitudinal mid-sagital section was also estimated. The ratio of medulla to cortical thickness is one indicator of the species' urine concentrating capacity (Munkacsi and Palkovits, 1965). The glomerular diameter was calculated as D = L+B/2, where, L is the long glomerular axis and B is the short glomerular axis. The glomerular volume was calculated using the formula $GV= \pi/6(LB)^{3/2}$, where GV is the glomerular volume, π is a constant equal to 3.14, L is the long glomerular axis and B is the short glomerular axis. The relative glomerular blood volume (RGBV) was calculated as described by Palkovits and Zolani (1963) using the formula RGBV = GV X N, where, GV is the glomerular volume and N is the average number of glomeruli. Relative medullary thickness (RMT) was calculated according to the method of Sperber (1944) as RMT = 10 (medullary thickness) / $(\text{Kidney volume})^{1/3}$, where kidney volume is the product of the dimensions of the kidney.

Analyses of Malondialdehyde (MDA) and Reduced Glutathione (GSH):

Colourimetrically, lipid peroxidation was estimated by measuring thiobarbituric acid reactive substances. This method is based on 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 (Yoshioka *et al.*, 1979). The concentration of GSH was estimated according to the method of Ellman (1959). This method is based on the reduction of 5, 5'-Dithiobis-2-nitrobenzoic acid with GSH to yield a yellow color, which is spectrophotometrically measured at 412 nm.

Assays of Catalase (CAT) and Superoxide Dismutase (SOD):

Catalase activity was determined according to the method of Xu *et al.* (1997). Superoxide dismutase (SOD) was estimated according to the method of Kakkar *et al*. (1984). This method is based on inhibiting the composition of NADH-phenazine methosulphate nitroblue tetrazolium formazan. The color formed at the end of the reaction can be extracted into butanol and spectrophotometrically measured at 560 nm.

Immunohistochemical Investigations:

Kidneys 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 mouse Bcl-2 and p53 antibodies at a dilution of 1:1000 and 1:400, respectively, (Dako Pty Ltd., Campbellfield, VIC, Australia). The 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 rinsed using DAB (3, 3'-Diaminobenzidine) for 3 min as brown color then stopped with distilled water. The sections were countered stained with hematoxylin, dehydrated in ascending grades of alcohol, cleared and mounted. The positive cells numbers were counted in 30 fields which were randomly selected from three different sections in each region using X 40 objective lens.

Statistical Analysis:

The results are expressed as the mean±SD. Comparison between the studied regions was carried out using the Independent Samples Test. The recorded $p<0.05$ were considered to be statistical significance. All statistical analyses were performed using SPSS statistical version 20.0 software package (SPSS® Inc., USA).

RESULTS

Data presented in Table 1 show the average body weight, kidney weight and the ratios of the kidney (KBW) to the bodyweight of the lesser Egyptian gerbil, *G. gerbillus* from North Coast and Faiyum regions. The body weight and kidney weight were significantly different $p < 0.05$ and recorded 27.34±3.69 g and 0.11±0.01 g for North Coast region, respectively, and 18.32±3.63 g and 0.09±0.01 g for Faiyum region, respectively (Table 1).

Table 1: Body and kidney weights of *G. gerbillus* from North Coast and Faiyum regions

Items	Dabaah, North Coast	Kom O'Shim, Faiyum
Body wt (g)	27.34*±3.69	18.32*±3.63
Kidney wt (g)	0.11*±0.01	0.09*±0.01
Kidney wt /Body wt x 10^{-3}	3.93±0.56	5.30±1.15

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

Hematological and Biochemical Parameters:

There was a significant increase in WBCs, platelets, hemoglobin, MCV, MCHC and eosinophils of *G. gerbillus* from North Coast region when compared with those from Faiyum region. There was a significant increase in RBCs only of *G. gerbillus* from Faiyum region when compared with those from North Coast region (Table 2).

Table 2: Hematological parameters of *G. gerbillus* from North Coast and Faiyum regions.

Items	Dabaah, North Coast	Kom O'Shim, Faiyum
RBCs x 10^6/µl	3.89*±0.35	5.46*±0.48
WBCs x 10^3/µl	3.50*±1.49	2.38*±0.89
Platelets x 10^3/mm^3	285.25*±104.77	291.25*±56.09
Hb (g/dl)	11.83*±1.32	10.85*±1.69
Hct (%)	37.66±3.94	37.96±6.34
MCV (fl)	91.51*±3.58	85.05*±4.05
MCH (pg)	31.38±1.35	30.56±1.45
MCHC (g/dl)	33.05*±1.26	30.23*±1.08
Lymphocytes (%)	43.75±5.28	43.13±8.43
Monocytes (%)	2.00±0.76	2.13±0.64
Neutrophils (%)	47.25±5.63	49.38±12.37
Eosinophils (%)	2.13*±0.83	1.88*±0.64

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

All tested biochemical parameters were significantly different $p<0.05$ except serum creatinine, uric acid and chloride of *G. gerbillus* from North Coast and Faiyum regions. The only maximum values of sodium 154.76±1.17 (mmol/l) were recorded for

North Coast region. The maximum values of Urea 67.38±10.57 (mg/dl), cholesterol 218.25±43.91 (mg/dl), triglycerides 206.88±32.05 (mg/dl), potassium 6.79±0.14 (mmol/l), calcium – total 10.31±0.09 (mg/dl), calcium – ionized 5.20±0.20 (mg/dl), phosphate 27.49±1.43 (mg/dl) and magnesium 4.63±0.13 (mg/dl) were recorded for Faiyum region (Table 3).

Table 3: Biochemical parameters of *G. gerbillus* from North Coast and Faiyum regions

Items	Dabaah, North Coast	Kom O'Shim, Faiyum
Urea (mg/dl)	54.26*±5.15	67.38*±10.57
Creatinine (mg/dl)	1.54±0.33	1.34±0.03
Uric acid (mg/dl)	2.41±0.68	3.26±0.13
Cholesterol (mg/dl)	157.50*±14.51	218.25*±43.91
Triglycerides (mg/dl)	157.38*±16.48	206.88*±32.05
Potassium (K) (mmol/l)	6.55*±0.19	6.79*±0.14
Sodium (Na) (mmol/l)	154.76*±1.17	143.25*±1.28
Chloride (Cl) (mmol/l)	113.46±1.16	110.13±1.96
Calcium – total (mg/dl)	2.90*±0.08	10.31*±0.09
Calcium – ionized (mg/dl)	1.42*±0.10	5.20*±0.20
Phosphate (PO4) (mg/dl)	12.25*±1.16	27.49*±1.43
Magnesium (Mg) (mg/dl)	4.19*±0.08	4.63*±0.13

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

Histological Observations:

There was a significant difference $p < 0.05$ between *G. gerbillus* from North Coast and Faiyum regions in all average values of the kidney (Fig. 2) parameters measured in this study (Table 4). The highest average value of kidney volume 209.25±67.70 (mm^3) was recorded for North Coast and the lowest value was 174.00±52.19 (mm^3) for Faiyum region. The highest average value of cortical thickness 1.02±0.14 (mm) was recorded for North Coast region and the lowest one was 0.69±0.09 for Faiyum region. The whole medullary thickness was 1.11 (mm) for North Coast region and 1.05 (mm) for Faiyum region. The RMT was 2.31±0.25 for North Coast region and 1.92±0.23 for Faiyum region. The superficial glomerular number, diameter, volume and RGBV were 102.50±6.61, 60.75±8.63 (µm), 11.29±4.21 (μ^3) and 11.49±4.13 for North Coast region respectively. The superficial glomerular number, diameter, volume and RGBV were 93.75±4.80, 51.89±7.59 (µm), 7.34±3.14 (μ^3) and 7.39±3.18 for Faiyum region respectively. The juxtamedullary glomerular number, diameter, volume and RGBV were 72.50±4.14, 73.55±8.84 (µm), 20.13±6.43 (μ^3) and 14.69±5.13 for North Coast region respectively. The juxtamedullary glomerular number, diameter, volume and RGBV were 52.13±5.79, 72.90±7.36 (µm), 20.29±6.43 (μ^3) and 10.79±4.50 for Faiyum region, respectively, (Table 4).

Table 4: Kidney measurement parameters of *G. gerbillus* from North Coast and Faiyum regions.

Items	Dabaah, North Coast	Kom O'Shim, Faiyum
Kidney Volume (mm^3)	209.25*±67.70	174.00*±52.19
Cortical Thickness (mm)	1.02*±0.14	0.69*±0.09
Outer Medullary Thickness (mm)	0.43*±0.01	0.42*±0.02
Inner Medullary Thickness (mm)	0.68*±0.13	0.63*±0.07
Relative Medullary Thickness (RMT)	2.31*±0.25	1.92*±0.23
Superficial Glomerular Number	102.50*±6.61	93.75*±4.80
Superficial Glomerular Diameter (μm)	60.75*±8.63	51.89*±7.59
Superficial Glomerular Volume x 10^4 (μ^3)	11.29*±4.21	7.34*±3.14
Superficial RGBV x 10^6	11.49*±4.13	7.39*±3.18
Juxtamedullary Glomerular Number	72.50*±4.14	52.13*±5.79
Juxtamedullary Glomerular Diameter (μm)	73.55*±8.84	72.90*±7.36
Juxtamedullary Glomerular Volume x 10^4 (μ^3)	20.13*±6.43	20.29*±6.43
Juxtamedullary RGBV x 10^6	14.69*±5.13	10.79*±4.50

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

Oxidant/Antioxidant Biomarkers:

The results of all oxidative stress parameters show significant changes $p<0.05$ were determined in the levels of the kidney MDA, GSH, CAT and SOD between the two studied regions. The gerbils from North Coast showed a significant increase $p < 0.05$ in the values of the kidney GSH, SOD and CAT when compared with those from Faiyum region. The gerbils from Faiyum region showed a significant increase in the values of the MDA in the kidney 21.52±1.21 mmol/g fresh weight when compared with those from North Coast region. Also, a significant decrease of the gerbils' kidneys in the values of GSH, SOD and CAT were observed for the gerbils from Faiyum region (Fig. 3).

Immunohistochemical Investigations:

Examination of the gerbils' kidney sections from North Coast region showed weak expression of the Bcl-2 anti-apoptotic protein (25% of the cells, stained) while positive strong immunohistochemical expression of bcl2 as indicated by the brown colour (61-100% of the cells, stained) were observed for those from Faiyum region. A weak positive immunohistochemical expression of the pro-apoptotic protein p53 was observed for gerbils from North Coast region while moderate positive expression of the p53 (26-60% of the cells, stained) for those from Faiyum region was observed (Fig. 4).

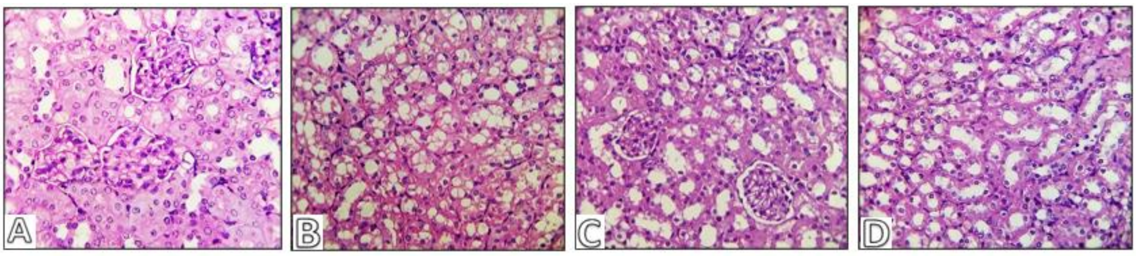

Fig. 2: Photomicrograph of *G. gerbillus* kidney from North Coast (A & B) and Faiyum (C & D) regions, H & E stain (X 400).

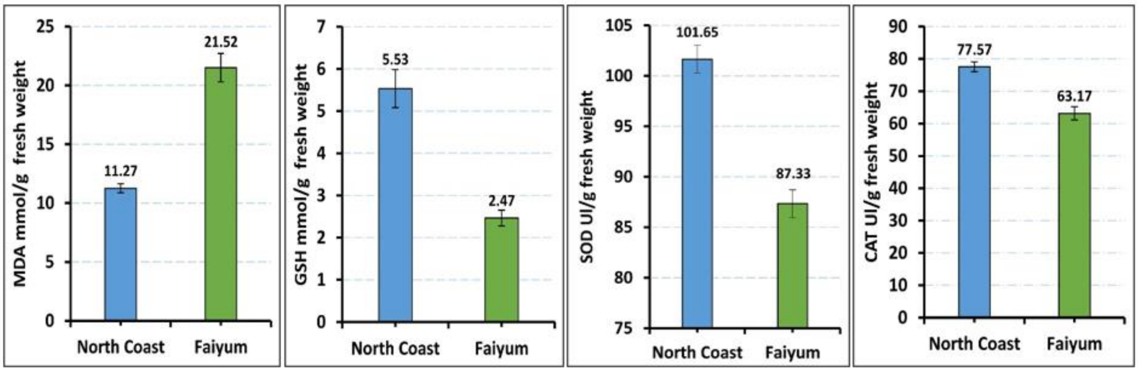

Fig. 3. Kidney oxidant/antioxidant biomarkers in *G. gerbillus* from North Coast and Faiyum regions. The values represent mean±SD, significantly different $p < 0.05$. MDA: malondialdehyde, GSH: reduced glutathione, SOD: superoxide dismutase and CAT: catalase.

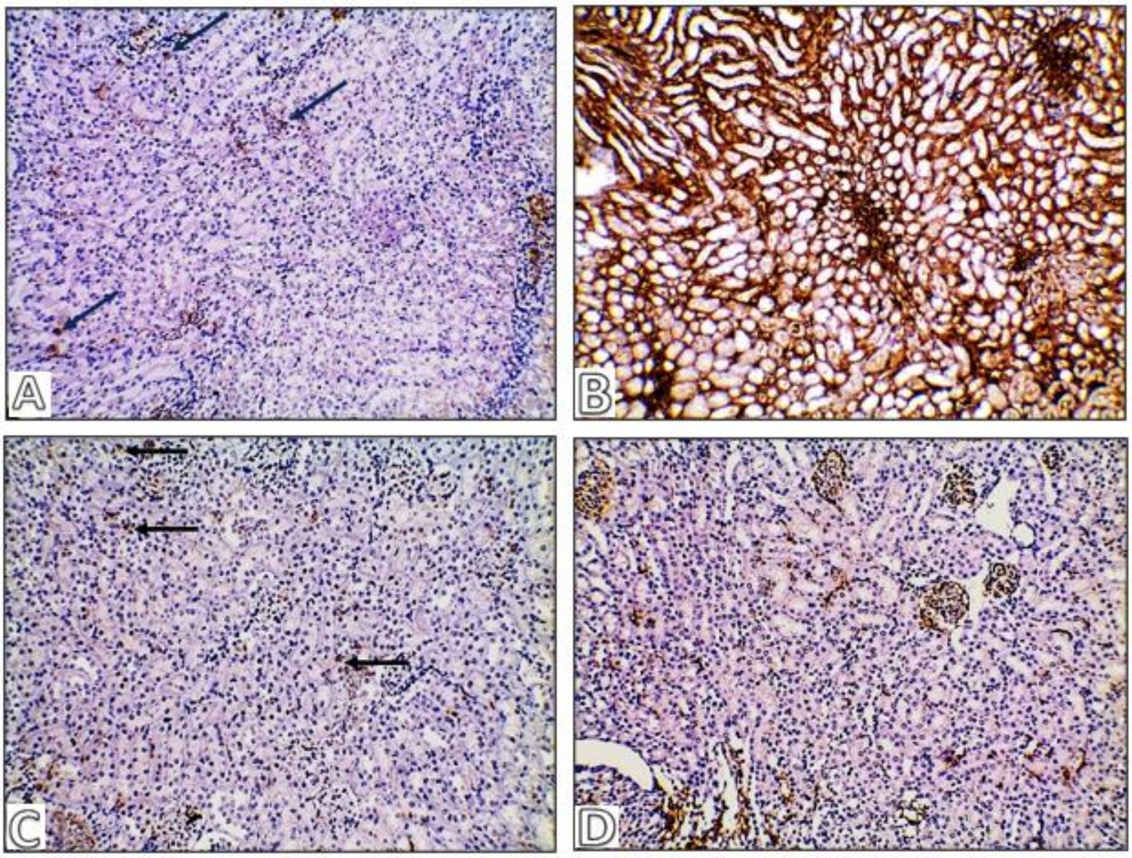

Fig. 4. Light photomicrographs of the gerbils' kidney sections immunostained with anti-apoptotic protein Bcl-2 and pro-apoptotic protein p53 (magnification 200 X). (A) North Coast region showing weak Bcl-2 expression (25% of the cells, stained) (blue arrows), (B) Faiyum region showing positive strong immunohistochemical expression of Bcl-2 as indicated by the brown colour (arrows) (61-100% of the cells, stained), (C) North Coast region showing that weak positive immunohistochemical expression of the pro-apoptotic protein p53 (25% of the cells, stained) (black arrows) and (D) Faiyum region showing moderate p53 expression (26-60% of the cells, stained).

DISCUSSION

The lesser Egyptian gerbil, *G. gerbillus*, is a small yellowish-orange gerbil with ear and sole not pigmented. Tail is bicolored; brush moderately prominent, grayish to brownish. White supraorbital and postauricular markings and white rump patches are conspicuous. The ear length is less than one-half of hindfoot length. The posterior margin of the nasals is round or pointed, the skull has large tympanic bulla, palatine foramina relatively long, and incisive foramina relatively short. These findings were in agreement with Osborn and Helmy (1980).

The present study is in agreement with Osborn and Helmy (1980) who mentioned that *G. gerbillus* inhabits coastal dunes of white nummulitic limestone sand in Western Mediterranean coastal belt. Adjacent to and sometimes in salt marshes. Sandy areas within the coastal vegetation. In Faiyum it inhabits sandy patches in cultivated areas, palm groves and sandy areas of semi-desert supporting reeds (*Phragmites australis*), grasses such as *Stipagrostis scoparia* and *Panicum turgidum* and shrubs such as *Heliotropium digynum* (Yunker and Guirgis, 1969).

The Western Mediterranean coastal belt is the richest part of Egypt in its floristic composition owing to its relatively high rainfall. *G. gerbillus* feeding on seeds, leaves and stems of some plants found in this region. The number of species in this belt makes up about 50% of the total Egyptian flora which is estimated to be about 2000 species (Oliver, 1938), 2080 species (Täckholm, 1974), 2094 species by Boulos (1995). Boulos (2000; 2002; 2005) recorded a total of 2125 species of which 50 species are cultivated. Most of these species flourish during the winter season and are called therophytes. Zahran and Willis (2009) documented that the floristic species of the Western Mediterranean coastal belt enjoy good climatic conditions than those of the other parts of Egypt. There are more species and several numbers of individual plants and the vegetation are more or less continuous, not like that in the inland desert areas. In the autumn numerous geophytes make an attractive show of flowers and in late spring grasses and members of the Compositae, Leguminosae, and Cruciferae are somewhat abundant (Zahran and Willis, 2009).

The plant communities are separated by large stretches of barren ground between the Western Mediterranean coastal belt and the Faiyum Depression which is considered an extension of the Nile Valley. The vegetation in the Faiyum Depression includes trees of *Acacia raddiana* and *Phoenix dactylifera* there are bushes undershrubs and grasses, such as *Zygophyillum album, Tamarix* spp., *Nitraria retusa, Fagonia Arabica, Desmostachya bipinnata* and *Alhagi maurorum*. Common xerophytes can also be seen in the desert surrounding the Faiyum Depression. Zahran and Willis (2009) reported that *Nitraria retusa*, *Alhagi graecorum* and *Desmostachya bipinnata* are restricted to the interdune habitat. Four species, namely: *Tamarix nilotica, Phoenix dactylifera, Sporobolus spicatus* and *Imperata cylindrical* are growing in most regions of Faiyum. The lesser Egyptian gerbil feeding on some of these vegetations.

The present results showed that gerbils from North Coast region significantly increased in WBCs, platelets, hemoglobin, MCV, MCHC and eosinophils while those from Faiyum region were significantly increased in RBCs count only. The only maximum values of sodium were recorded for gerbils from North Coast region whereas the gerbils from Faiyum region recorded the highest values of urea, cholesterol, triglycerides, potassium, calcium – total, calcium–ionized, phosphate and magnesium were recorded for Faiyum region. Old *et al.* (2005) mentioned no differences in hemoglobin levels between three species of murids from the Australian desert. The age of an animal can affect the levels of some hematological and biochemical parameters (Moustafa, 1997; Riviello and Wirz, 2001). However, the difference of habitat types is not possible to make any significant comments on the hematologic and biochemical parameters measured in this study. Alternatively, the gerbils tested in this study are desert-dwelling species and have developed special adaptive strategies (such as high-water conservation strategies and nocturnal lifestyle) to subsist in dry habitats (Baverstock and Watts, 1974; Baverstock and Green, 1975).

In the present results, the levels of chloride and sodium in serum were significantly higher than in published ranges for other mammals, including humans, a marsupial (Viggers and Lindenmayer, 1996), the porcupine (Moreau *et al.,* 2003) and the pouched rats (Nssien *et al.,* 2002). However, serum chloride and sodium levels in this study were quite comparable to another wild rodent, the dusky-footed woodrat (Weber *et al.,* 2002), a Mustelid (Thornton *et al.,* 1979) and the Hyrax (Aroch *et al.,* 2007).

The present results showed a significant difference between gerbils from North Coast and Faiyum regions in the values of cortical and medullary thickness, RMT, superficial and juxtamedullary glomerular numbers, diameters, volumes and RGBV. The all-average glomerular number of gerbils' kidney from North Coast region were 175 glomeruli greater than those from Faiyum region which were 145.88 glomeruli.

In the kidney of gerbils from North Coast and Faiyum regions there was a great variation in size between glomeruli. The average diameter of juxtamedullary glomeruli was greater 1.21 times in gerbils from North Coast and 1.40 times in gerbils from Faiyum than superficial glomeruli. The average volume of juxtamedullary glomeruli was 1.78 and 2.76 times greater than superficial glomeruli in gerbils from North Coast and Faiyum regions, respectively. This led to preferential filtration in these nephrons and because they have long loops, and this may result in maximal concentrating capacity. Similar observations were recorded by Hanssen (1961) and Basuony (1997). Munkacsi and Palkovits (1965) stated that the juxtamedullary glomeruli were 1.69 bigger than the cortical glomeruli in semidesert animal, *Galago senegalensis*, 1.01 in desert animal, *Jaculus jaculus*, and 0.28 in water requiring animal, *Rattus norvigicus*. The variation between superficial and juxtamedullary glomeruli was the first adaptation that had been observed in the kidney of desert rodents by Munkacsi and Palkovits (1965). This variation in size leads to suspect that the glomerular filtration surface would be much greater in the large juxtamedullary glomeruli.

Sperber (1944) was the first to join the length of the loop of Henle to urine concentrating ability in mammals. According to this model, the maximum urine concentrating ability is related directly to the length of the loops of Henle and collecting ducts that traverse the renal medulla and inversely to a nephron’s diameter. In mammals, Schmidt-Nielsen and O’Dell (1961) demonstrated the quantitative correlation between the relative length of the Henle's loops, as reflected by relative medullary thickness, and the maximum urine concentration.

According to the previous observations, the kidney of gerbils from North Coast region had greater filtration capacity than those from Faiyum region. This finding agrees with Hanssen (1961) who stated that the glomerular filtration rate of the juxtamedullary nephron is approximately eight times that of nephron from the outer cortex. The variation in the number of glomeruli in the kidney provides a direct influence on the ability to produce concentrated urine, and also was agreed with Abdalla and Abdalla (1979).

One of the adaptations of desert rodents is an increased elongation of the inner medullary portion of the kidney. In the present results, the inner medullary thickness of both gerbils from North Coast was 0.68±0.13 mm and Faiyum region was 0.63±0.07 mm, this result agrees with the gross adaptation of desert species especially rodents (Basuony, 1993). The RMT was significantly increased in gerbils' kidneys from North Coast than those from Faiyum region. The relative thickness of the medulla was related to the animal habitat. The lengthening of the renal papillae is associated with that of the collecting ducts and therefore results in an increased area for water reabsorption via passive back diffusion under the influence of antidiuretic hormone (Schimdt-Nielsen and O'Dell, 1961; March and Segel, 1971).

The present data revealed that gerbils from North Coast region showed a significant increase in the values of the kidney GSH, SOD and CAT whereas the gerbils' kidneys from Faiyum region showed a significant increase in the values of the MDA only.

Zhu *et al.* (2019) reported that superoxide is effective in preventing and treating diseases related to superoxide free radicals. When SOD anion radicals are produced excessively or the SOD concentration is low, excessive superoxide anion will cause oxidation. Reduced glutathione is an important antioxidant in mammals that can scavenge the free radicals in cells and decrease the damage of the cell membrane caused by the forming of reactive oxygen species via lipid peroxidation (Berndt and Lillig, 2017). Also, Ighodaro and Akinloye (2018) mentioned that the activities of superoxide dismutase, catalase and glutathione peroxidase comprise a first-line antioxidant defense system. Moreover, Karabacak *et al.* (2015) reported that lipid peroxidation considers a well-defined mechanism of cellular damage in animals. The increase in the lipid peroxides perhaps result from a decrease in antioxidant status and increased production of free radicals. Meanwhile, Kim *et al.* (2011) found that lipid peroxides come apart to form more complex and reactive compounds such as MDA and 4-hydroxynonenal which consider indicators of cellular oxidative stress. GSH is a non-enzymatic antioxidant because of the direct interaction of its –SH group with reactive oxygen species (ROS). GSH may also involve in the enzymatic detoxification of reactive oxygen species as a coenzyme (Janssen *et al.,* 1993). This is explained why kidney GSH levels decreased in the gerbils from Faiyum region when compared with those from North Coast region in this study, as well as, the decline of SOD and CAT activities in the gerbils from Faiyum region. It is clear that SOD is a metalloenzyme and had the ability to accelerate the dismutation of endogenous cytotoxic superoxide radicals to hydrogen peroxide. This hydrogen peroxide is harmful to both the polyunsaturated fatty acids and the structural proteins of the plasma membrane. Whereas, CAT is responsible to remove H_2O_2 which was produced by the SOD (Karabacak *et al.,* 2015).

The present results indicated that gerbils from North Coast region showed weak anti-apoptotic protein expression of the Bcl-2 in kidney tissues and showed weak positive immunohistochemical expressions of the p53. The gerbils from Faiyum region showed positive strong anti-apoptotic protein expression of the Bcl-2 in kidney tissues and showed moderate positive immunohistochemical expressions of the p53. This may be due to the desert nature of the pristine environment in which the gerbils live in North Coast region. While Faiyum region is considered an environment with several urbanizations. Rotimi *et al.* (2016) showed that there were genomic alterations that may be associated with significant increases in the level of p53 and caspase-3 activity in rats. Liao *et al.* (2014) reported an increase in the expression of pro-apoptotic p53, Bax, and caspase-3 and a decrease in the expression of the anti-apoptotic Bcl-2 levels in ducklings' tissues.

CONCLUSIONS

It was noted that the gerbils from North Coast have kidneys that are more efficient than those that live in Faiyum region. Although the glomerular diameter and volume in the gerbils' kidneys from Faiyum region were larger than those from North Coast region unless the glomerular number and relative medullary thickness in gerbils' kidneys from North Coast region were greater than those from Faiyum region. The gerbils from North Coast region showed a significant increase in the values of the kidney

GSH, SOD and CAT whereas the gerbils' kidneys from Faiyum region showed a significant increase in the MDA values only. The gerbils from North Coast region showed weak anti-apoptotic protein expression of the Bcl-2 and weak immunohistochemical expressions of the p53 in kidney tissues. The gerbils from Faiyum region showed strong anti-apoptotic protein expression of the Bcl-2 and showed moderate immunohistochemical expressions of the p53 in kidney tissues.

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ARABIC SUMMARY

تركيب ووظائف الكُلى والإجهاد التأكسدي والتعبير الكيميائي المناعي لجربلس جربلس من موئلين مختلفين في مصر

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تمتلك الثدييات القاطنة لموائل قاحلة سُمك لُب نسبي أكبر وتركيز للبول أقصى من تلك التي تعيش في موائل بها وفرة من الماء والغذاء. يقطن الجربوع المصري الصغير، جربلس جربلس، الكُثبان الساحلية المتكونة من رمال الحجر الرملي الأبيض في الحزام الساحلي الغربي للبحر الأبيض المتوسط. وفي الفيوم، يتواجد هذا الجربوع في البقع الرملية للمناطق المزروعة وأشجار النخيل والمناطق الرملية المدعمة بالغطاء النباتي شبه القاحلة. ولقد استخدمنا 27 عينة حية من الجربوع المصري الصغير (14 من منطقة الساحل الشمالي و13 من منطقة الفيوم). وأظهرت النتائج أن الجرابيع من منطقة الساحل الشمالي لديها كُلى أكثر كفاءة من تلك التي تعيش في منطقة الفيوم. وعلى الرغم من أن قُطر وحجم الكُبات في كُلى الجرابيع من منطقة الفيوم أكبر منها في كُلى الجرابيع من منطقة الساحل الشمالي إلا أن عدد الكُبات في كُلى الجرابيع من منطقة الساحل الشمالي أكثر منها في كُلى الجرابيع من منطقة الفيوم. وسجلت النتائج أن السُمك الكلي للُب في كُلى الجرابيع من منطقة الساحل الشمالي 1.11 مليمتر بينما كان 1.05 مليمتر في كُلى الجرابيع من منطقة الفيوم. وكان السُمك النسبي للُب 2.31±0.25 لكُلى الجرابيع من منطقة الساحل الشمالي وكان 1.92±0.23 لكُلى الجرابيع من منطقة الفيوم. وأظهرت النتائج أن الجرابيع من منطقة الساحل الشمالي بها زيادة معنوية في قيم الكُلى للـ GSH وSOD وCAT في حين أن قيم الكُلى للجرابيع من منطقة الفيوم أظهرت زيادة معنوية في قيم الكُلى للـ MDA فقط. كما بينت النتائج أن الجرابيع من منطقة الساحل الشمالي ذات تعبير ضعيف في التعبير الكيميائي المناعي لكل من Bcl-2 وp53 في أنسجة الكُلى. بينما أظهرت الجرابيع من منطقة الفيوم تعبيراً قوياً في التعبير الكيميائي المناعي لـ Bcl-2 ومتوسطاً لـ p53 في أنسجة الكُلى.