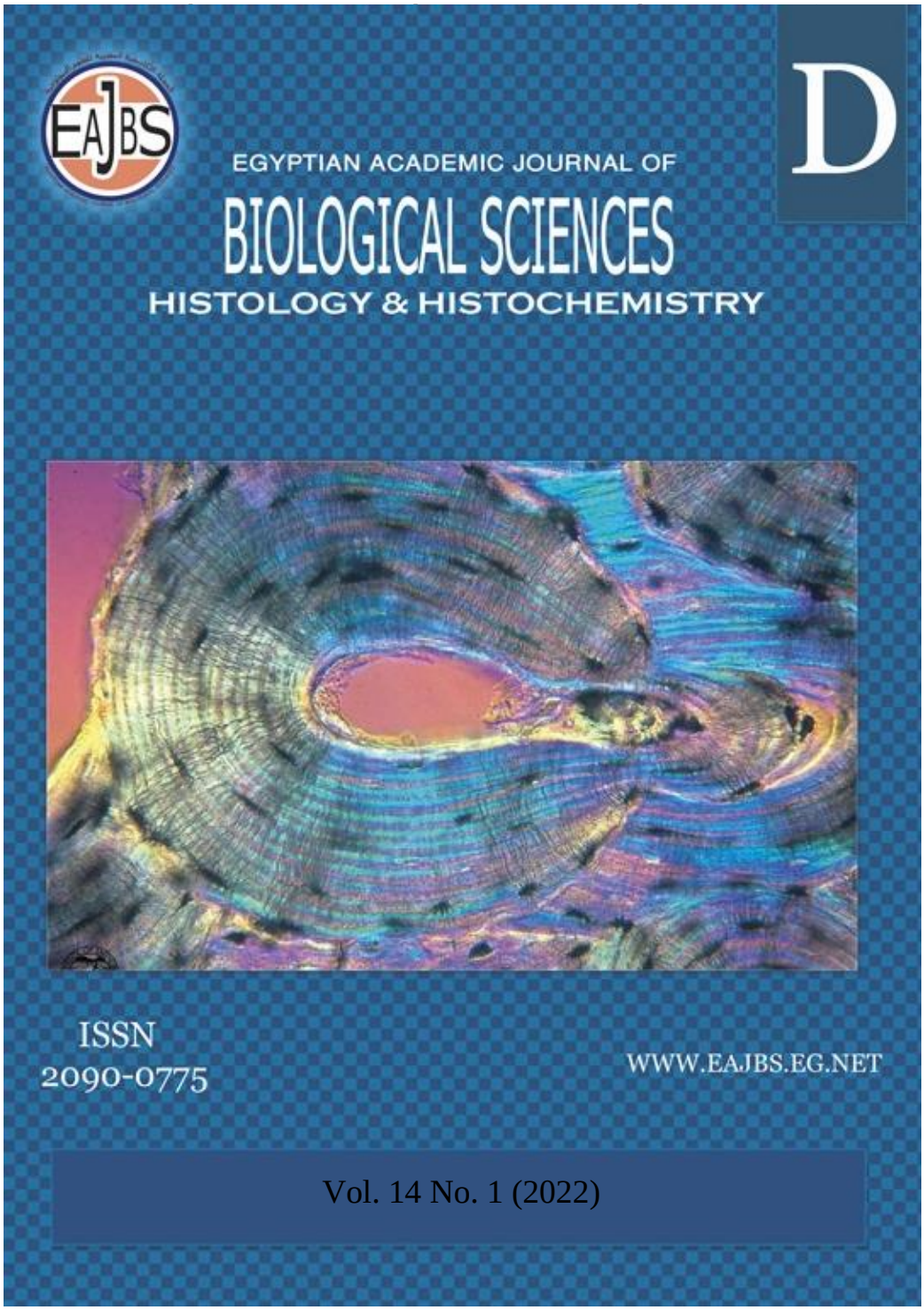

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Correlation of Renal Histopathological Changes, Lymphocytes Infiltration, Caspase-3 and Apoptosis Activation with Diabetic Nephropathy Induced by High Energy Diet in An Experimental Rat Model "*Psammomys obesus*"

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ABSTRACT

Aim of the work: The aim of our study is to investigate the effect of a high-energy diet on renal histopathological changes, T and B lymphocytes infiltration and expression levels of caspase-3 in apoptotic kidney cells of *Psammomys obesus*, an animal model that intimately mimics human diabetic complications. ***Material and methods:*** **A**fter 6 months of feeding with the high energy diet, metabolic parameters were evaluated using an enzymatic colorimetric kit, the renal histopathological changes were examined by histological methods, whereas the expression levels of lymphocyte cells and caspase-3 were evaluated with immunohistochemical methods. To compare the expression levels of caspase-3, T and B lymphocytes in control and experimental animals groups, we quantified immunohistochemistry images using free software ImageJ Fiji. ***Result:*** We have demonstrated that after diabetes induction, rats develop similar symptoms of human metabolic pathology, such as hyperglycemia, hyperlipidemia, renal histopathological changes associated with expression levels of renal caspase-3 and lymphocytes infiltration leading to renal dysfunction. ***Conclusion*:** Consequently, in this study, we proposed that histopathological changes are correlated with apoptosis activation and lymphocyte cells infiltration leading to kidney dysfunction. Thus, our model may be useful to better study and understand diabetes and its complications. This original valuable model will furthermore permit testing several new therapeutic strategies to prevent diabetes including diabetic nephropathy.

INTRODUCTION

The high energy diet is a major risk factor for the development of type 2 diabetes mellitus, **(**Capcarova e*t al*., 2018) a metabolic disease with a silent evolution and severe consequences leading to microvascular complications such as diabetic retinopathy, diabetic neuropathy and diabetic nephropathy **(**Nguyen *et al*., 2012). Diabetic nephropathy is the primary single cause of health problems associated with heavy socioeconomic burden and high morbidity and mortality rates worldwide **(**Ritz *et al*., 1999). It is typically characterized by glomerulosclerosis, glomerular hypertrophy, accumulation of extracellular matrix (ECM), thickening of basement membrane and renal inflammation (Ma *et al*., 2014). once the renal parenchyma is inflamed, interstitial and glomerular inflammatory cells such as macrophages, neutrophils, fibrocytes, masts cells and T and B lymphocytes infiltrate the tissue and produce many profibrotic cytokines and growth factors which enhance fibroblast accumulation and ECM production (Hou *et al*., 2005). It has been shown that infiltration of T and B lymphocytes in renal parenchyma was the first event of renal fibrosis initiation (Pillai, 2019)**,** displaying pathogenic function that leads ultimately to interstitial, glomerular and tubular atrophy. The process with which renal parenchyma loses these cells is commonly called apoptosis (Burne *et al.*, 2001).

Apoptosis known as programmed cells death is a physiological regulatory process playing an essential role in organism growth and tissue homeostasis. However, this process is deregulated during a variety of human pathology (Barnes et *al*., 1998) including diabetic nephropathy (Verzola *et al*., 2004).

It has been reported that the apoptosis process was controlled by a variety of signal cascades called caspases (for cysteinyl aspartate-specific proteinase) (McIlwain *et al*., 2013)**.** This endoprotease has a crucial role in preserving tissue homeostasis through regulating apoptosis and inflammation. Caspases involved in the activation of apoptosis are classified by their mechanism of action in two groups, the first group called initiator caspases and the second called executioner caspases (Mariathasan *et al*., 2004). Of these cysteine proteases, caspase-3 is a member of the executioner caspases serving as cytoplasmic regulators of apoptosis, inflammation and fibrosis in renal parenchyma (Yang *et al*., 2001). Remarkably, caspase-3 inactivation attenuated interstitial inflammation, decreased glomerular and interstitial extracellular matrix deposition and protected from diabetic nephropathy (Shahzad *et al*., 2016)**.** This suggested that caspase-3 is believed to be a therapeutic target to prevent renal fibrosis, interstitial inflammation and attenuate diabetic nephropathy.

Despite, the high prevalence of diabetic nephropathy, a variety of hypothesis have been suggested concerning the development of this pathogenesis. But there are no studies to date evaluating the correlation between renal structural changes, renal lymphocytes infiltration and caspase-3 expression in human diabetic nephropathy.

Understanding this correlation leads to the development of effective targeted therapies and the prognosis of patients with diabetic nephropathy. Our hypothesis is that diabetic nephropathy manifested as a modification on kidney structure is related to lymphocytes infiltration and caspase-3 activation. To validate this hypothesis, we have performed histopathological and immunohistochemical analysis on *Psammomys obesus*, a well-known animal model for diabetes research (Lahfa *et al*., 1995)**,** which faithfully reproduces the human diabetic

pathology (Marquie et *al*., 1984). We have demonstrated that after induction of diabetes with a high-energy diet, *P.obesus* presents similar symptoms of human diabetic nephropathy. This pathogenesis is associated with renal structural changes leading to inflammatory responses characterized by T and B lymphocytes infiltration and expression levels of caspase-3 in apoptotic kidney cells of the animal model *Psammomys obesus*. Therefore, we proposed that T and B lymphocytes infiltration, caspases-3 expression, renal structural damages are interlinked on diabetic nephropathy. Thus, understanding each process at a suitable time is essential to testing potential therapeutic and pharmacologic approaches for the prevention of human diabetic nephropathy.

MATERIALS AND METHODS

Experimental Animals:

Sixteen adult sand rats *P. obesus*, weighing between 96.16g and 154.56g were collected in the Beni-Abbes arid area (Wilaya of Bechar, Algeria; 30°7′N, 2°10′W) of the Sahara Desert. *P. obesus* is a diurnal species that live in the Sahara Desert of North Africa, and feeds on halophil plants of the *Chenopodiaceae* family (*Suaedamollis*, *Traganumnudatum*, and *Salsolafoetida*) (Daly and Daly, 1973). Animals were adapted to laboratory conditions (25°C, 70% hygrometry, and 69 rounds of 12-hour light-dark cycles) and after a period of acclimation (2 weeks), during which all animals were fed on a natural diet (ND): *Salsolafoetida* (20kcal/day), they were randomly distributed into two groups:

- The first group (n=8) was nourished on ND (50g/day/animal, equivalent to 30-32 kcal). The halophilic plants represented a low caloric diet
-The second (n=8) group were nourished with a standard laboratory diet that represented a high-energy diet (HED), (15–20 g/day/animal, equivalent to 52–70 kcal) and allowed free access to saltwater (NaCl 0.9%) (El Aoufi *et al.*, 2007). At the end of the experiment (6 months), all *P. obesus* were sacrificed.

All experiments were ethically performed according to the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA), following approval by the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research. The permits and ethical rules were achieved according to the Executive Decree n˚ 10–90 completing the Executive Decree n˚04–82 of the Algerian Government, establishing the terms and approval modalities of animal welfare in animal facilities. Furthermore, it was recently supported by the local university ethical committee of the "Association Algérienne des Sciences en Expérimentation Animale" AASEA (Agreement Number 45/DGLPAG/DVA.SDA.14).

Analytical Methods:

During the experimental period which lasted 6 months and in order to monitor changes in body weight, animals were weighed every month during the 6 months of the experiment. For metabolic monitoring, the blood was collected every month in heparin tubes from the retroorbital sinus of the eye using a Pasteur pipette (Bouguerra *et al*., 2004). At the end of the experiment (6 months), animals were sacrificed after anesthesia by intraperitoneal injection of urethane (25%) at a rate of 0.4 ml/100 g body weight and blood plasma was separated by centrifugation (3000 rpm) for 10 min and used for the estimation of glucose, triglycerides, and total cholesterol. The glucose content was determined by the oxidase-peroxidase (GOD-POD) enzymatic method (Trinder, 1969). The plasma triglycerides and the total cholesterol were estimated, respectively, by the enzymatic method using the Monozyme diagnostic kit (Fossati and Prencipe, 1982), and the Siedel method (Siedel *et al*., 1983).

Histopathological Study:

At the end of the experimentation (6 months) animals of both groups were sacrificed and kidneys were fixed in formalin, dehydrated using alcohol series and embedded in paraffin the kidney was cut to a thickness of 5 µm (Martoja and Martoja-Pierson, 1967)**.** Kidney sections for *P. obesus* fed with ND or with HED were stained with Hematoxylin-Eosin (HE), Masson's Trichrome (MT) and Periodic Acid–Schiff (PAS).

Immunohistochemical Analysis:

Immunohistochemistry was carried out using the peroxidase-labelled Streptavidin-Biotin Technique (Van Noorden, 1990). For detection of lymphocytes cells infiltration, two antibodies were used: anti-CD3 (for T lymphocytes cells detection) and anti-CD20 (for B lymphocytes cells detection) mouse monoclonal antibodies (Cell brand, USA). However, an anti-caspase-3 rabbit monoclonal antibody (Cell Signaling, Beverly, MA, USA) was used for the detection of apoptosis. Paraffin sections (5 µm) were deparaffinized, hydrated and incubated in hydrogen peroxide (3% in PBS) for 10 min to block endogenous peroxidase activity. The antigens were retrieved by boiling for 20min in citrate buffer (tri-sodium dehydrate, MERCK Millipore) at pH 6.0, using a microwave, followed by cooling to room temperature for 20 min. After washing in PBS, different sections were incubated with primary antibodies (anti-CD3, anti- CD20 and anti-caspase-3), applied overnight at 4° C in a humid atmosphere. Then the sections were washed in phosphate-buffered saline before incubation with Anti-Mouse biotinylated secondary antibody (BA9200, anti-mouse biotinylated, Vector Laboratories) for 25 min at room temperature. After washing the slides were incubated with labeled avidin-biotin-peroxidase (Vectastain Elite ABC Peroxydase kits universal, Vector, PK-6100) for 30 min at room temperature. Then the slides were washed and revealed with 3, 30-diaminobenzidine peroxydase substrate (kit DAB Vector, SK-Vector Laboratories). Finally, sections were counterstained with hematoxylin and mounted in Glycergel (Dako, Glostrup, Denmark). The slides were observed using a photonic microscope (Motic SFC-18) equipped with a camera (hirocam, 5 megapixels) and connected to a laptop.

To compare the expression levels of caspase-3, CD20 and CD3 of the two animal groups (control and experimental), we quantified immunohistochemistry images by converting the original images into RBG images which were deconvolved by ImageJ using the color deconvolution plugin (free software ImageJ Fiji version 1.2; WS Rasband, National Institute of Health, Bethesda, MD), https://imagej.net/Fiji/Downloads.

Statistical Analysis:

All results are expressed as the mean value ± standard error of the mean value (S.E.M.) and were analyzed statistically with Student's test. Calculations were performed using statistical analysis software (Statistica 6.0; StatSoft, France). Differences were considered significant when $P < 0.05$.

RESULTS

Bodyweight Results:

The body weights in *P. obesus* reared on a natural diet remained more or less stable throughout the entire period of experimentation, whereas the body weights in animals fed on a high energy diet significantly increased since the third month (Fig.1A). ($P< 0.05$).

Biochemical Results:

During the experimental period (6 months), the blood glucose level, plasma cholesterol and triglyceride evolution of *P. obesus* nourished on ND remained more or less at the same level**.** Whereas for the animal group nourished on HED, the plasma glucose, cholesterol and triglyceride levels were significantly increased during the

experimental period (Fig. 1B, 1C, 1D). ($P<0.05$).

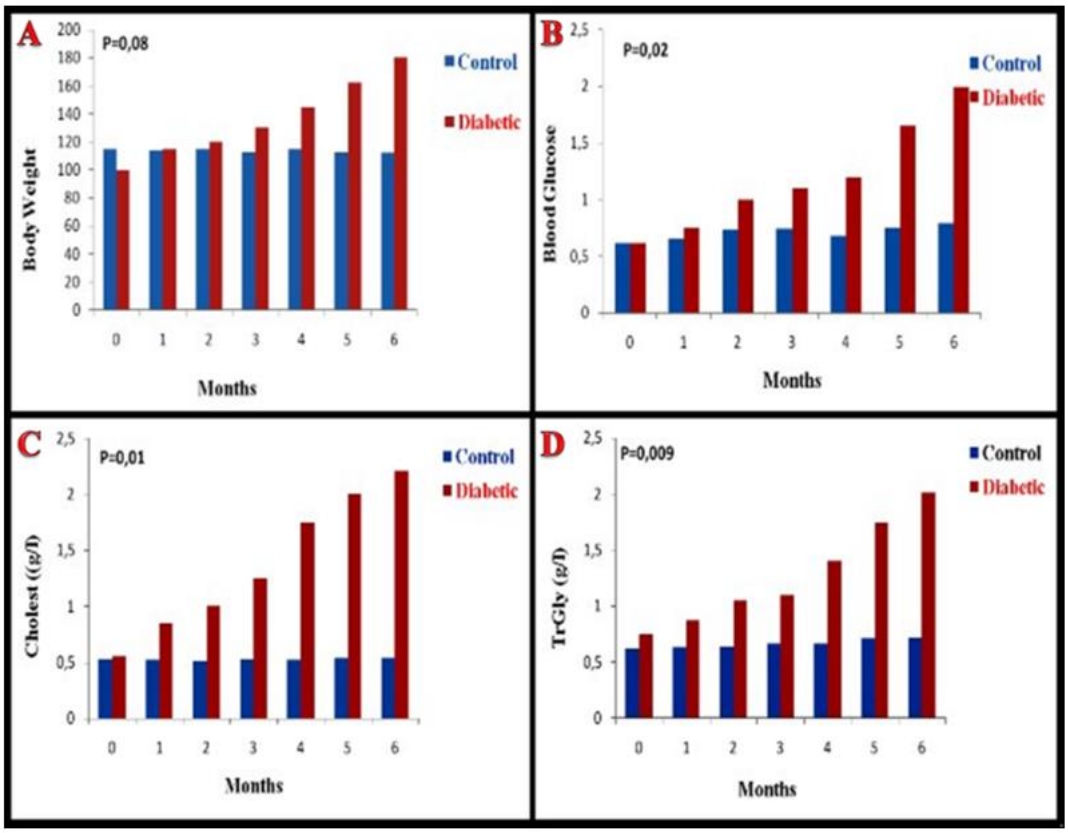

Fig. 1 Body weight and metabolic parameters evolution in *P.obesus*.
Since the Psammomys (n=8) received ND the body weight, blood glucose level, cholesterol and triglyceride remained more or less at the same level during experimental period (blue bars), animals (n=8) nourished on HED (red bars) showed a significant increase in body weight, blood glucose level, plasma cholesterol and triglyceride. Data are expressed as mean ± standard deviation.

Histopathological Results:

The kidney tissue section of *P.obesus* reared with ND showed a normal morphological structure of the glomerulus and renal tubules (Fig. 2A, 3A and 4A). In contrast, kidney sections of *P.obesus* nourished on HED appeared several structural changes in renal parenchyma characterized by tubular atrophy, interstitial inflammation leading to interstitial fibrosis and expansion of bloods vessels, (Fig. 2B, 2C, 2D, 3B, 3C, 3D, 4C and 4D) which inducing hemorrhage apparition (Fig. 3B 3C and 3D). The glomerular capillaries were destroyed and the Bowman's space was dilated (Fig. 2C, 2D, 3C and 3D). Simultaneously severe glomerular damages appeared in the kidney section of the diabetic animal model such as glomerular tip lesion (represented by yellow arrow) distinguished by adhesion between the glomerular tuft and Bowman's capsule (Fig. 2D and 2C). Other histopathological glomerular lesions were observed in kidney sections of diabetic *P. obesus* characterized by glomerular vacuolization associated with deterioration of glomerular mesangial matrix (represented by arrow-heads) (Fig. 2D). While, section kidney of *P. obesus* stained with Periodic Acid Schiff demonstrated various renal structural changes that occur in the glomerulus and the tubulointerstitium associated with hyalines deposits (Fig. 4B, 4C and 4D) leading to arterial intimal thickening (Fig. 4B). Periodic

acid Schiff appeared severe glomerular lesions distinguished by glomerular crescent. This histopathological damage is associated with a rupture of the glomerular basement membrane and hyperplasia of parietal epithelial cells (Fig. 4D).

Immunohistochemical Results:

Kidney sections of the control group immunostained with anti-Caspase 3 revealed no staining in glomerulus and tubules (Fig. 5A). In contrast, kidney section of *P.obesus* submitted to HED for 6 months showed a positive expression of caspases-3 enzyme in nuclear area cells of proximal and distal tubules with no immuno-staining in glomerular regions (Fig. 5B, 5C, 5D). The representative staining intensity of the expression of caspase-3 is shown in (Fig. 5E). The caspase-3 expression is significantly higher in kidney parenchyma of *P.obesus* nourished on HED, compared with the kidney section of animals submitted on a natural diet. However, kidney sections labeled with anti-CD3 (T lymphocytes cells) and anti-CD20 (B lymphocytes cells) showed no staining in control groups throughout the experiment. This observation allowed us to the absence of inflammatory response in this group (Fig. 6A and Fig. 7A). In contrast, the kidney section of animals nourished on HED showed positive immunostaining in the whole of kidney parenchyma (Fig. 6B, 6C, 6D, 7B, 7C and 7D). The staining intensity of CD20 and CD3 expression was significantly higher in the kidney section of *P.obesus* nourished on HED compared to the control group (Fig. 7E).

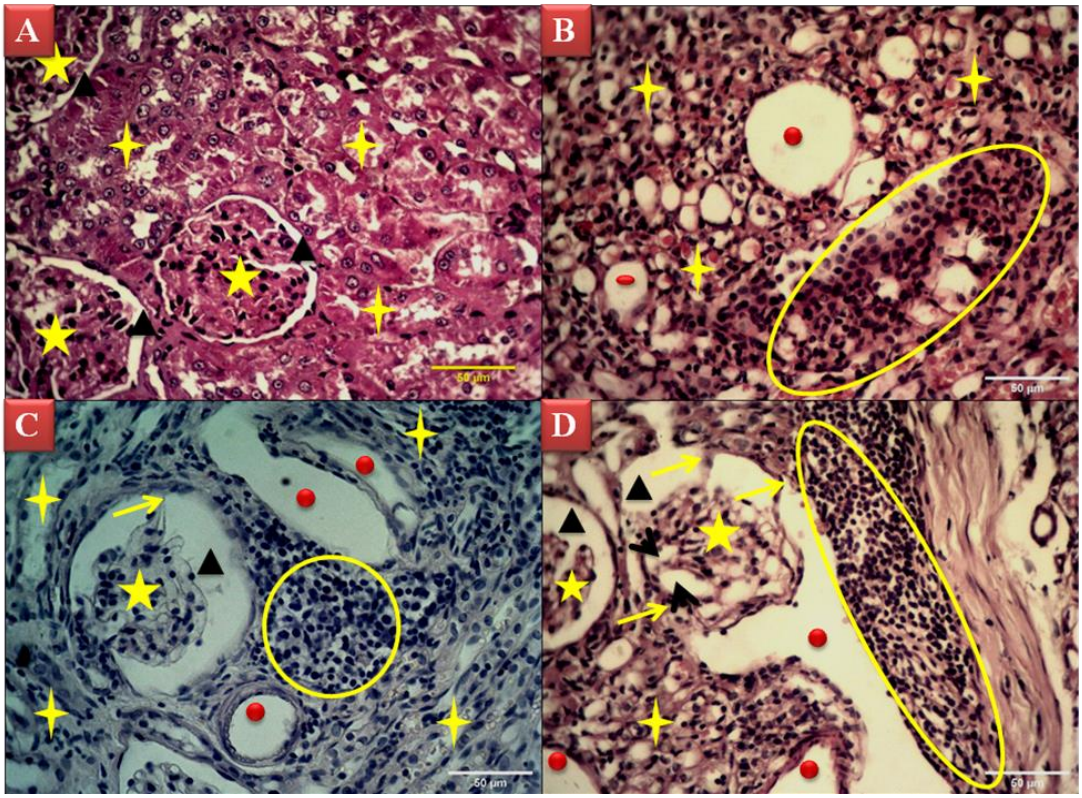

Fig. 2 Histopathological analysis of kidney stained with Hematoxylin Eosin.
Histological section of non diabetic *P. obesus* reared ND **(A)** showed intact structural of renal parenchyma characterized by normal glomerulus encircled by Bowman's space and by small number of blood vessels. Interstitial space presented the organized proximal and distal tubules with absence of inflammatory process. Section kidneys of *Psammomys obesus* fed with HED showed an important number of dilated blood vessels, accompanied by focal interstitial inflammation, tubular atrophy (**B, C and D).** The glomerular capillaries were destroyed and the Bowman's space was dilated. Severe glomerular alterations are observed such as tip lesion (**yellow** arrow **in C and D)** and glomerular capillaries lumen dilatation (arrow-heads in **D)** Star correspond to glomerulus, filled circles to blood capillary, empty-circles to focal interstitium inflammation, cross to proximal and distal tubes and triangle to the Bowman's space. Scale bar 50µm.

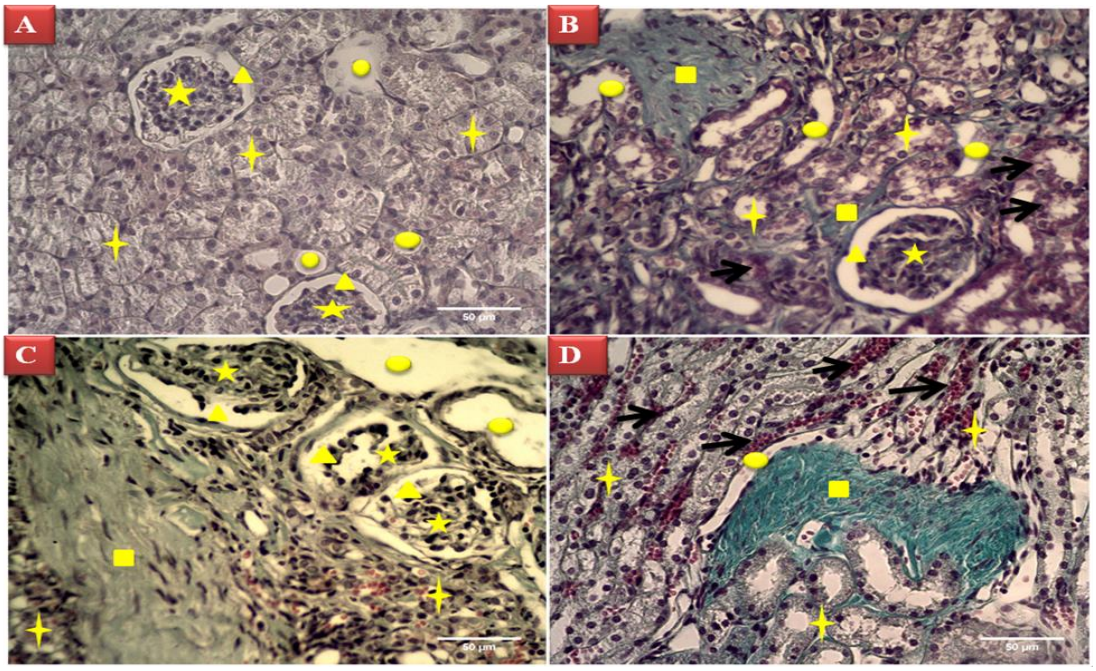

Fig. 3 Histopatological Characterization of kidney stained with Masson's Trichrom. Kidney section of non diabetic *Psammomys* consumed ND **(A)** does not show any extracellular matrix deposits appearing the normal architecture of the glomerulus and renal tubules. Whereas, section kidneys of *Psammomys obesus* fed with HED (**B, C, and D)** showed considerable interstitial fibrosis **(square in B, C and D),** tubular atrophy **(cross),** dilated lumen of blood vessels **(filled circles)** and increased hemorrhage **(arrow in D).** We observed inflammatory cells situated between tubules and inside the glomerulus. Glomerular capillaries were destroyed **(stars in C)** and the Bowman's space was dilated **(triangle in B and C)**. Scale bar 50µm.

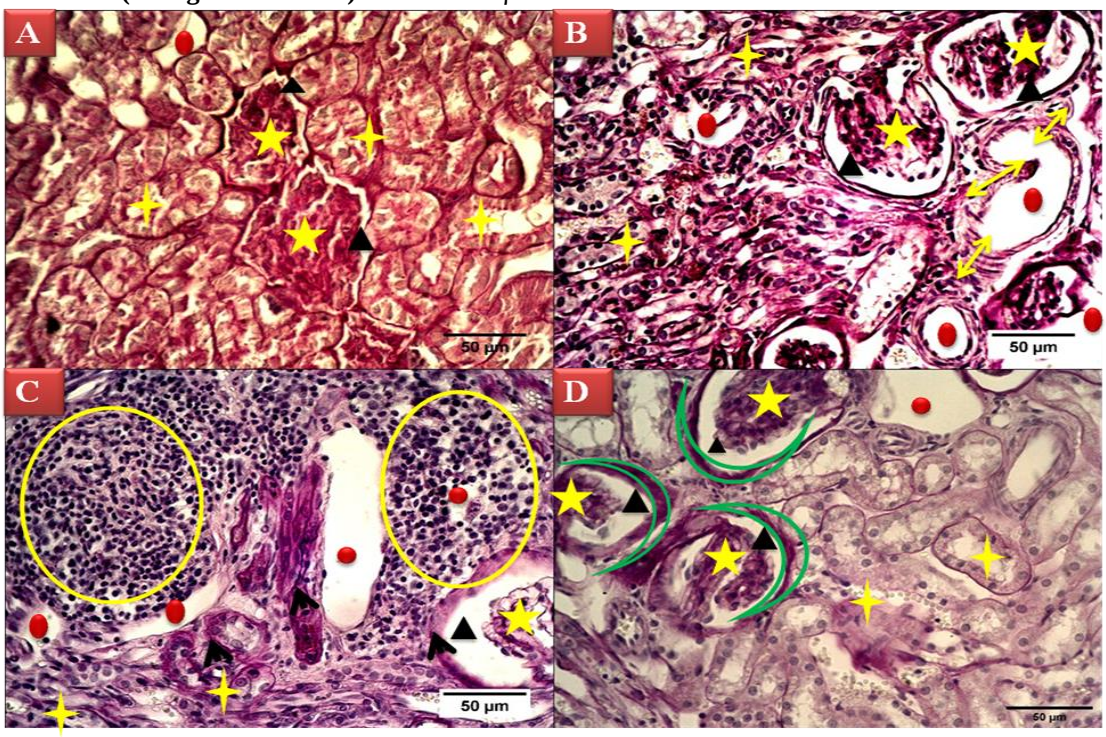

Fig. 4 Histopatological Characterization of *Psammomys'* kidney using PAS staining. Section kidney of *P.obesus* nourished on ND **(A)** does not show renal structural lesions. However, Section kidney of sand rat consumed HED **(B, C, and D)** showed severe renal lesions characterized by Atherosclerosis indicated by hyaline deposits **(arrow-heads in C)** leading to arterial intimal thickening **(double ended arrow in B).** Focal interstitial inflammation **(empty-circles in B, C and D)** induces disorganize and denature tubules **(cross in C and B)** with dilated bloods vessels **(filled circles in B, C and D).** Severe glomerular damage evidenced by epithelial crescent **(green crescent in D).** Scale bar 50µm.

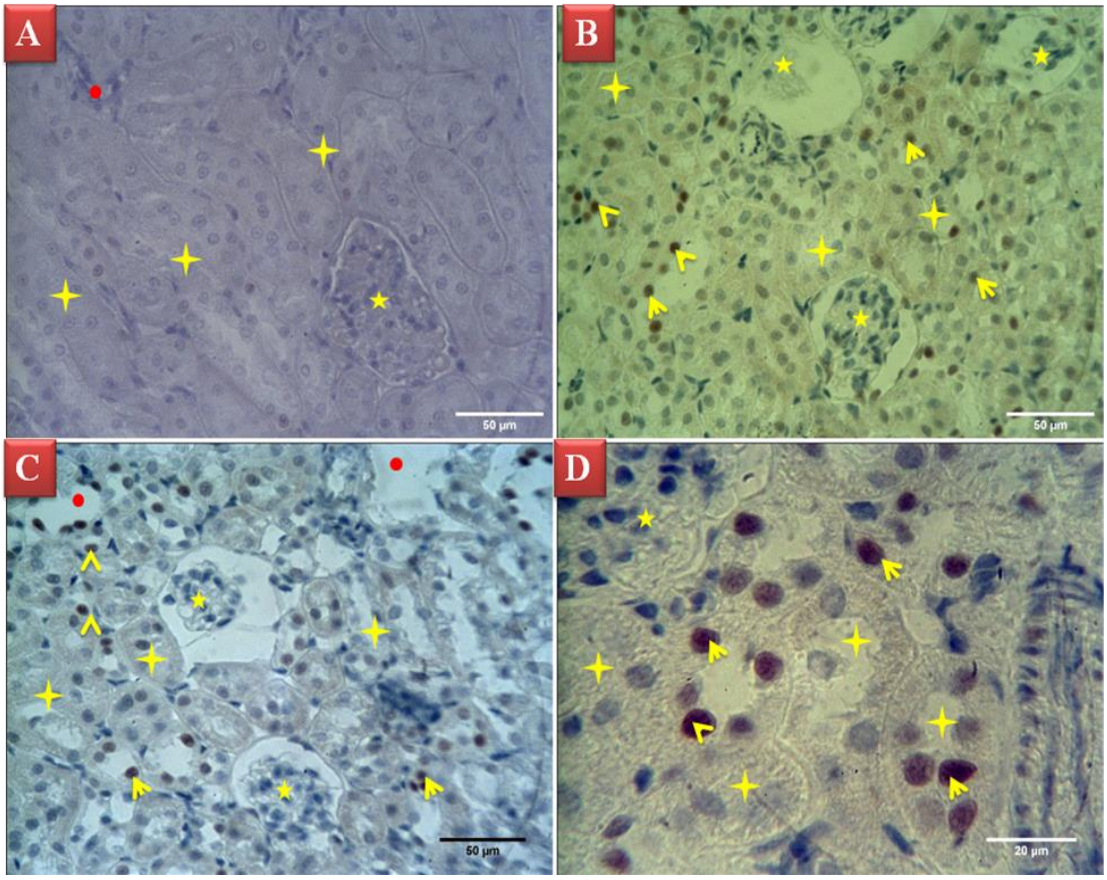

Fig. 5 Immunohistochemical staining of caspase-3 in Psammomys' kidney.
Caspase-3 is not expressed in the kidney of animals nourished with ND **(A)**. Since the 6 months of HED **(B and C)** positive caspase-3 immunostaining **(illustrated by arrow-heads)** was revealed in kidney parenchyma of *Psamommys*, situated in nuclear area cells of proximal and distal tubules with no immuno-staining in glomerular regions (stars).

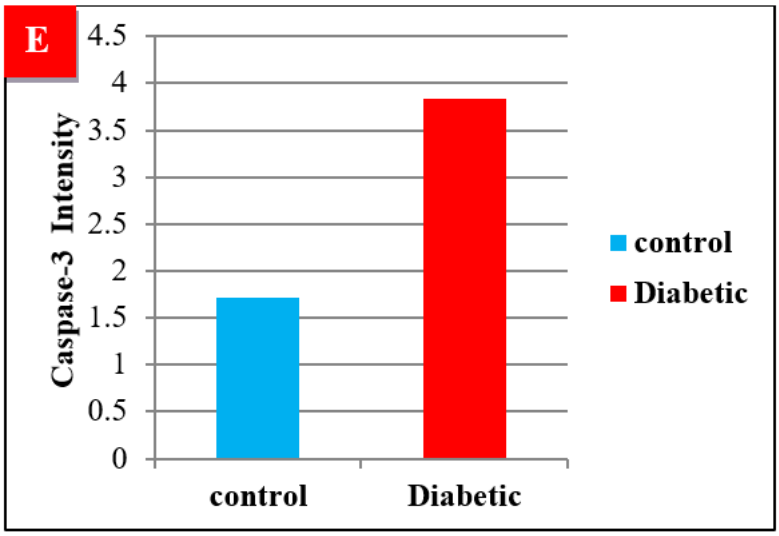

Fig 6. The quantitative analysis of caspase-3 expression in control and diabetic rats.
The quantitative analysis of caspase-3 expression revealed a significant increases of caspase-3 intensity in the kidney section of *P.obesus* nourished with HED **(red bar in E)** compared to the control group **(blue bar in E)** which caspase-3 intensity was feebly expressed**.** Values are mean ± SEM, P<0.001. Scale bar 50 µm and 20 µ.

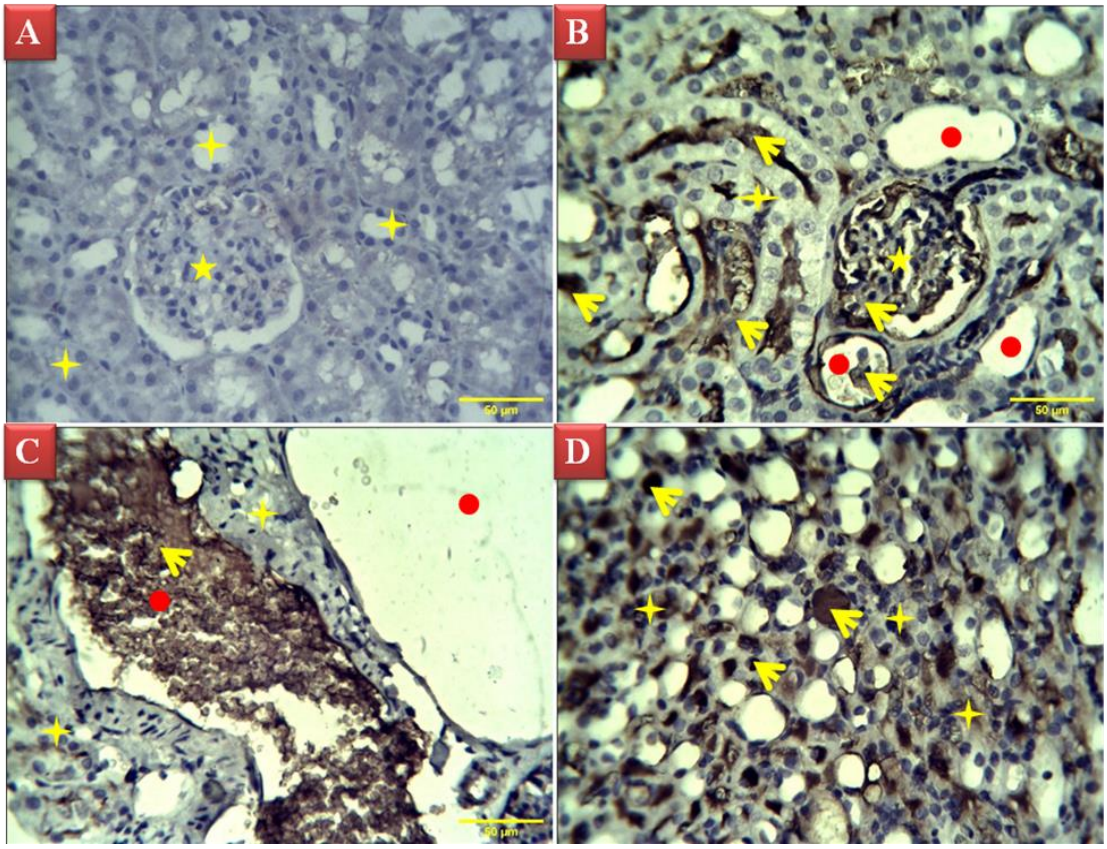

Fig. 7 immunohistochemical labeling of CD20 (B lymphocytes cytoplasmic and membranes) in Psammomys' kidney.
CD20 antibody was weakly positive in control groups **(A)**, in contrast kidney section of diabetic groups revealed a significant increase in positively stained cytoplasms and membranes of sclerotic glomerili, dilated or atrophied tubules and expended interstitium **(arrow in B, C and D).**

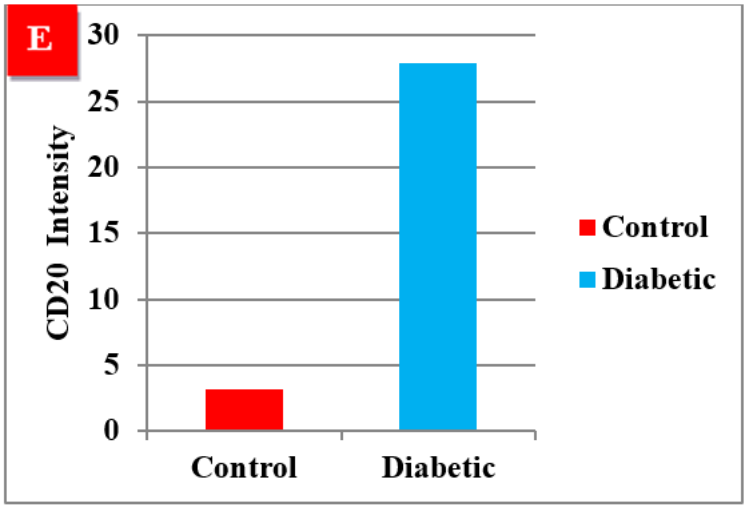

Fig. 8 The quantification of CD20 intensity in control and diabetic rats.
The quantification of CD20 intensity was significantly increases in kidney of diabetic *P.obesus* **(red bar in E)** compared to the control group **(bleu bar in E)**. Values are mean ± SEM, P<0.001. Scale bar 50 µm

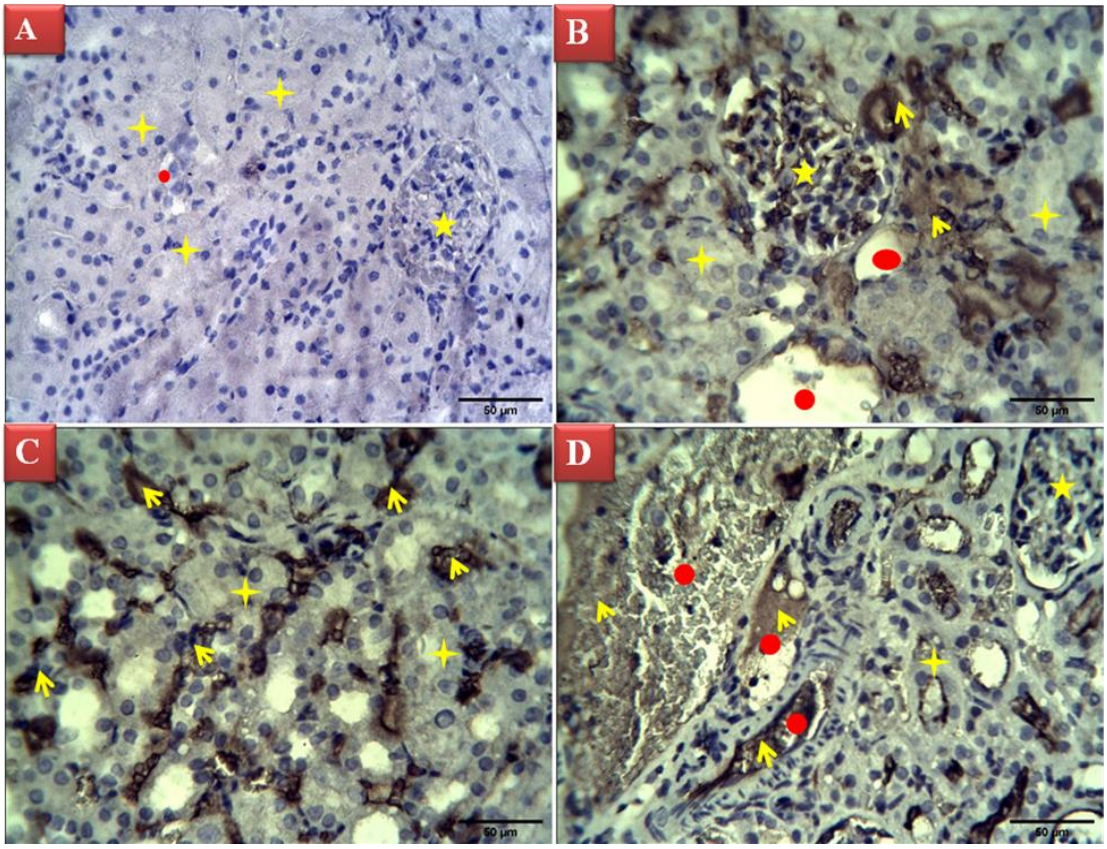

Fig. 9 immunohistochimical labeling of CD3 (T lymphocytes membranes) in Psammomys' kidney.
CD3 antibody was weakly positive in control groups **(A)**, in contrast kidney section of diabetic groups demonstrated a significant increase in positively stained membranes of glomerili, tubules, expended interstitium and dilated blood vessels **(arrow in B, C and D).**

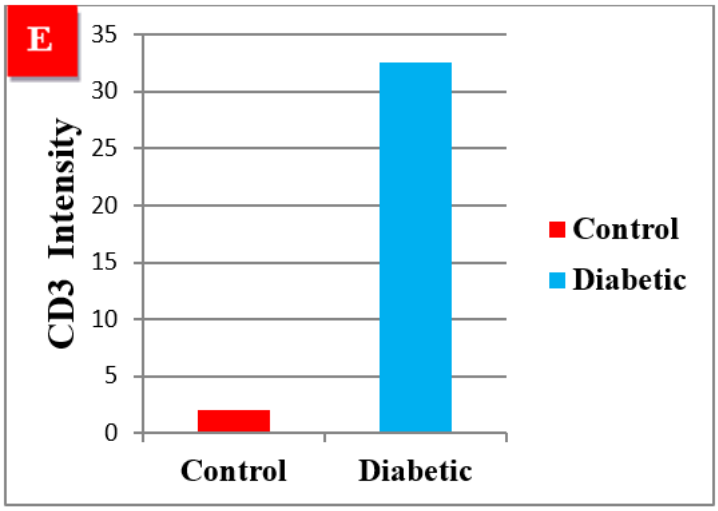

Fig. 10 The quantification of CD3 expression in control and diabetic rats**.**
The quantification of CD3 expression revealed significant increases of CD3 intensity in kidney of diabetic *P.obesus* **(red bar in E)** compared to the control group **(bleu bar in E)** Values are mean ± SEM, P<0.001. Scale bar 50 µm.

DISCUSSION

Although long (6 months) administration of HED is known to cause diabetes and renal tissue disorders in *P. obesus*, (Kalman *et al*., 1993)**,** the relationship between renal histopathological changes, lymphocytes cells infiltration and apoptosis in the kidney of an animal model which reproduces human diabetic nephropathy have not yet fully elucidated. Understanding this correlation is mandatory for the development of more efficient therapy against this pathology. In the present study, we focused on the correlation between these processes by using the animal model *P. obesus*, which develops similar human complications of small vessels such as diabetic nephropathy (Kalderon et *al*., 1986)**,** when is fed a high energy diet (HED). In agreement with several studies, our work demonstrates that *P. obesus* exposed to HED develop hyperglycemia, increases body weight, hypertriglyceridemia and hypercholesterolemia (Shafrir, 2001)**.** This hyperlipidemia stimulates others lipids mediators which decreases the activity of the matrix-degrading metalloproteinase leading to the accumulation of ECM proteins, mesangial expansion, and glomerulosclerosis (Proctor et *al*., 2006; Kanwar *et al*., 2011)**.** According to previous histopathological studies in human diabetic nephropathy, our kidney sections of *P.obesus* fed a HED show similar results such as tubular atrophy, Thickening of the tubular and glomerular basement membrane, interstitial fibrosis (An *et al*., 2015). In addition, our kidney sections of *P. obesus* fed a HED stained with PAS showed comparable human Microcardiovascular changes characterized by renal intimal arterial thickening which is causally linked to hyalinosis or mesangial expansion (Bell, 1953). It has been reported that renal intimal arterial hyalinosis reduces arterial wall and facilitates the development of hypertension and ischemia nephropathy. (Ralph *et al.*, 1991). Severe and serious alterations were examined in kidney sections of sand rat fed a HED such as glomerular tip lesion which was described as adhesion between glumerular capillary tuft and Bowman's capsule, (Mungan *et al*., 2015), this alteration appears one of the frequent causes of the end stage of renal failure (Maisonneuve *et al*., 2000). In agreement with other studies, our histopathological analysis in the kidney of *P.obesus* nourished on HED showed a hyperplastic lesion named glomerular crescent and defined as an unspecific disorder involving the circumference of Bowman's capsule (Bajema *et al*., 2018)**.** This alteration is the consequence of glomerular capillaries rupture inducing infiltration of immune cells and cytotoxic elements in renal parenchyma. (Chen *et al*., 2019). Glomerular capillaries of experimental *P. obesus* nourished on HED showing dilatation or vacuolization of their lumens. This process is secondary to degeneration of glomerular mesangial matrix (mesangiolysis) causing severe damage and kidney dysfunction (Cattell *and Bradfield*, 1977). On the other hand, glomerular epithelial crescent, glomerular tip lesion and glomerular capillaries vacuolization are described as unspecific and unknown disorders which induce excessive glomerular morphological changes leading to rapid renal damages and kidney dysfunctions (Bajema *et al*., 2018). In human glomerulopathies, these processes commonly represent a rare event. Surprisingly, we have observed these rare processes for the first time in the kidney of our animal model. This finding confirms that *P. obesus* is an excellent model to study human diabetes including diabetic nephropaty.

Renal histopathological changes are the consequence of activation and expression of pro-inflammatory cytokines, chemokines and adhesion molecules (Aroune *et al*., 2016).

Renal inflammation is the earliest response to renal parenchyma damage. It is characterized by infiltration of inflammatory cells such as macrophages, neutrophils, fibrocyte mast cells, T and B lymphocytes in the site of injury (Kurts *et al*., 2013). This is in agreement with our immunohistochemical staining (CD3 and CD20 staining) showing infiltration of the immune cells in kidney parenchyma of *P.obesus* fed a HED. This is in accordance with human and animal experimental studies which demonstrate that humoral (B lymphocytes) and cellular (T lymphocytes) immune cells play a key role in the initiation and propagation of renal lesions (Jang *et al*., 2010**).** Interestingly, it has been reported that CD20 and CD3 attenuated injury in the initial phase and conferred protection from kidney diseases (Lobo *et al*., 2012), including diabetic nephropathy (Lim *et al*., 2010**).** In contrast, when renal inflammation is prolonged both T and B lymphocytes cells produce proinflammatory cytokine and growth factors, which have a crucial role in the production and deposition of extracellular matrix (ECM) in renal tissue leading to glomerular and tubulointerstitial atrophy (Weller *et al*., 2017). The process with which renal parenchyma loses these cells is commonly called apoptosis. (Burne *et al*., 2001).

Renal apoptosis recognized as programmed cells death is a physiological process responsible for the regulation of cells number through the development of the organism (Koseki *et al*., 1992). Furthermore, this process is involved in glomerular and interstitial cells loss during the course of kidney diseases (Lorz *et al*., 2006). It has been reported that renal apoptotic pathways are induced by a variety of internal or external signals such as survival factor deficiency, mitochondrial damage, lysosomal deterioration and caspase cascade activation. (Sanz *et al*., 2008). The caspase cascade system has a critical role in the regulation, induction and execution of apoptosis (Launay *et al*., 2005) However, Caspases are a family of proteases that maintains homeostasis through controlling inflammation and cell death (Yang *et al*., 2001). Caspases have a principal role in the activation of the programmed cell's death **(**Elmore, 2007). Among them, caspase-3 is thought to be a fundamental enzyme having a key role in the execution of apoptosis (Thornberry *et al*., 1997). This is in accordance with our immunolabeling, showing a positive expression of caspase-3 in kidney tissue of diabetic *P.obesus* nourished with HED for 6 months. Previous studies showed that the localization of the immunostaining of caspase-3 in rat kidneys during the progression of kidney disease has proved difficult (Krajewski *et al*., 1997). Here, for the first time, our immunohistochemical staining in renal parenchyma of diabetic *P.obesus* showed the localization of a positive expression of caspase-3 in tubulointerstitial regions with no caspase-3 labeling in the glomeruli. This is in agreement with other works using the same caspase-3 antibody in the human kidney showing positive staining in interstitial regions with little or no caspase-3 staining in the glomerular space (Krajewska *et al*., 1997). This finding certified that *P.obesus* is an excellent animal model for human diabetic nephropathy research.

Since, our results and other works (Fujihara *et al*., 2000) involved caspase-3 in apoptosis-related with the progression of diabetic nephropathy, it is essential to indicate the correlation between caspase-3 activity, interstitial and glomerular inflammation, progression of fibrosis and evolution of diabetic nephropathy.

It has shown that caspase-3 deficiency protects mice from interstitial and glomerular inflammation, decreases renal fibrosis and attenuates diabetic nephropathy.

(Yang *et al*., 2001; Shahzad *et al*., 2016). Thus, caspase-3 is believed to be a therapeutic target to prevent renal fibrosis, interstitial inflammation and attenuates diabetic nephropathy**.** Consequently, knowledge of renal structural changes, lymphocytes infiltration and apoptosis in the kidney of *P.obesus*, an excellent animal model for diabetes research may suggest a novel strategy for the prevention and the treatment of diabetic nephropathy.

Conclusion

We have demonstrated that a long administration of HED modifies the plasmatic biochemical parameters characterized by hyperglycemia and hyperlipidemia with bodyweight increasing. These plasmatic blood level changes influence morphological and physiological renal parenchyma associated with ECM expansion which is considered as a common actor playing a critical role in the activation of immune system response and caspase-3 pathway which induce apoptosis process and kidney damages. Our study uses an excellent animal model of diabetes research which mimics completely the human diabetic complications. This research is the first demonstration of the correlation between diabetic nephropathy, renal structural changes; renal parenchyma inflammation associated with lymphocyte cells infiltration and expression levels of activated caspase-3 in apoptotic kidney cells of *P.obesus* after diabetes induction. Thus, our work opens the way to other studies to better understand the molecular and cellular mechanisms in order to ultimately permit the testing of potential therapeutic and pharmacologic approaches for the prevention of human diabetic nephropathy.

Abbreviations: DN: Diabetic nephropathy, ECM: extracellular matrix, HED: high energy diet, ND: natural diet, GBM: Glomerular Basement Membrane.

Conflict of interest: The authors declare that they have no competing interests.

Ethics Approval and Consent To Participate:

All experiments were ethically performed according to the guidelines of the Federation of European Laboratory Animal Science Associations (FELASA), following approval by the Institutional Animal Care Committee of the Algerian Higher Education and Scientific Research. The permits and ethical rules were achieved according to the Executive Decree n˚ 10–90 completing the Executive Decree n˚04–82 of the Algerian Government, establishing the terms and approval modalities of animal welfare in animal facilities. Furthermore, it was recently supported by the local university ethical committee of the "Association Algérienne des Sciences en Expérimentation Animale" AASEA (Agreement Number 45/DGLPAG/DVA.SDA.14).

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ARABIC SUMMARY

الارتباط بين التغيرات النسيجية المرضية الكلوية، تسلل الخلايا الليمفاوية، تفعيل كاسباس 3 وتفعيل موت الخلايا المبرمج مع اعتلال الكلية السكري الناجم عن نظام غذائي عالي الطاقة في نموذج الفئران التجريبي "*Psammomys obesus*"

جميلة أرون [1،2]، وهيبة بلوي [2]، اعميروش مرسلي [3] ، صوفي ليبوشر [4] ، سيرجيو ماركو [4،5]

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3 كلية العلوم، قسم الفلاحة، جامعة امحمد بوقرة، بومرداس - الجزائر.

3 معهد كوري، مركز الأبحاث، جامعة الأبحاث - فرنسا.

5 جامعة باريس جنوب، جامعة باريس ساكلاي ، أورساي - فرنسا.

هدف الدراسة: الهدف من دراستنا هو التحقيق في تأثير النظام الغذائي عالي الطاقة على التغيرات النسيجية الكلوية، وتسلل الخلايا الليمفاوية T و B ومستويات التعبير عن الكاسباس 3 في موت الخلايا المبرمج في الكلى عند *Psammomys obesus* ، وهو نموذج حيواني يحاكي بشكل وثيق مضاعفات مرض السكري عند الإنسان.

المواد والطرق المستخدمة: بعد 6 أشهر من التغذية بالنظام الغذائي عالي الطاقة، تم تقييم معاملات التمثيل الغذائي باستخدام مجموعة قياس الألوان الأنزيمية، وتم فحص التغيرات النسيجية المرضية الكلوية بالطرق النسيجية، في حين تم تقييم مستويات التعبير عن الخلايا الليمفاوية و الكاسباس 3 بالطرق الكيميائية المناعية. لمقارنة مستويات التعبير عن الخلايا الليمفاوية و الكاسباس 3 في مجموعات الحيوانات الشاهدة والتجريبية، قمنا بتحديد صور الكيمياء المناعية باستخدام البرنامج المجاني ImageJ Fiji.

النتائج: لقد أثبتنا أنه بعد تحريض مرض السكري، تظهر على الجرذان أعراض مماثلة لأمراض التمثيل الغذائي البشري ، مثل ارتفاع السكر في الدم ، فرط شحميات الدم ، التغيرات النسيجية الكلوية المرتبطة بمستويات التعبير عن الكاسباس3 الكلوي وتسلل الخلايا الليمفاوية مما يؤدي إلى اختلال وظيفي كلوي.

الإستنتاج: نتيجة لهذه الدراسة اقترحنا أن التغيرات النسيجية المرضية مرتبطة بتنشيط موت الخلايا المبرمج وتسلل الخلايا الليمفاوية مما يؤدي إلى فشل الكلى. وبالتالي، قد يكون نموذجنا مفيدًا لدراسة وفهم مضاعفات مرض السكري بشكل أفضل. علاوة على ذلك، سيسمح هذا النموذج القيم الأصلي باختبار العديد من الاستراتيجيات العلاجية الجديدة للوقاية من مرض السكري بما في ذلك اعتلال الكلية السكري.

الكلمات المفتاحية: اعتلال الكلية السكري. بساموميس أوبيسوس. الآفات النسيجية المرضية. كاسباس .3 نظام غذائي عالي الطاقة.