



STUDY THE PATHO-PHYSIOLOGICAL CHANGES AFTER INDUCE DIABETES IN MOUSE

Muna Sachit Hashim¹ AL-Aamery¹, Layla Hashim Aloi² & Eman Hashim Yousif Atae³

^{1,3}Department of Pathology, College of Veterinary Medicine/University of Baghdad

²Department of Physiology, College of Veterinary Medicine/University of Baghdad

ABSTRACT

Diabetes mellitus induce by using alloxan which related to hyperglycemias' chemicals due to increase hepatic glucose. Alloxan consider as eta -cytotoxic that produced diabetes mellitus through targeting these cells of the pancreas by accumulates as glucose analogues, so causes low insulin release (Bhaskar, 2014). Diabetes is a chronic metabolite disorder conducts a major health concern today whose prevalence has continuously increased over worldwide in the past few decades. Diabetes mellitus consider as incurable metabolic disease that affects around 2.82% of the world people. Ankur (2012), there was poorly focusing in diabetes mellitus effects in different organs in mice such as skin, thymus, salivary gland and other organs, in spite of many studies were established in this concern. This study was designed to point the patho with physiological changes in different organs after induction diabetes in mice. The main histo-patho-physiological findings were elevated glucose in blood, fatty change, which appear as clear vacuoles of lipid pushed the nucleus a side fatty changes indicates the precipitation of lipid within tissues, evidence of necrosis and inflammatory infiltration in different organs, evidence of brain tissues damage, hyalinization of heart muscle, hyperkeratosis in esophagus and skin lesions as hair lost. Conclusion diabetes mellitus caused different pathophysiological changes in mice mainly increase glucose level in blood; appearance of fatty changes resulted from failure of liver to lipid metabolism. In addition, caused necrosis in pancreatic cell which responsible for insulin production and controlling the glucose and lipid in blood. Further to damage vital organs (brain and heart).

KEY WORDS: D.M. mouse; different organs.

INTRODUCTION

Alloxan is considered as toxic glucose analogue target to insulin-producing cells (beta cells) when injected to rodents. Insulin-dependent diabetes mellitus or D.M induced by alloxan is same to type I diabetes in humans^[1]. And work as specific toxicity for cells by accumulating within cytoplasm by increase uptakes GLUT² glucose transporter. That results in over producing of reactive oxygen species ROS associated to reduction of dialuric acid. These intracellular imbalances are initiates the toxicity to cells^[2]. Several experimental studies reveal that alloxan stimuli a sudden shock in insulin level in present or absent of glucose, which were occur yet alloxan injection^[3,4]. with full suppress to pancreatic cells response to glucose at low or high concentrates^[5] Besides alloxan action on pancreas' beta cells which propose as the important mark to determine alloxan diabetogenic similar status occurs through different reducing agents as glutathione; cysteine; ascorbic acid; sulfhydryl (-SH) groups^[6,7]. Alloxan acts with two-SH groups in the sugar-binding site of glucokinase results on form hard bond and in active form of enzyme then reduction in dialuric acid with highly value of ROS. superoxide radicals^[8,9]. These superoxide's radicals liberates ferrous ions from ferritin^[10]. Superoxide's radicals undergo modification results in release hydrogen peroxide H₂O₂. These ROS causes effects on the Deoxyribo Nucleic Acid of pancreas' islets. DNA damage stimulate poly ADP-ribosylation in which DNA repair. Anti-oxidizing agents must be used^[11] disturbances in intracellular calcium's ion homeostasis

were reported to be indicates induces' diabetes. Experimenters show that Calcium concentration become higher within beta cells of pancreas' islets^[12]. Calcium influxes are results from uncontrolled opens pores of calcium channels that's promoting calcium entrance in pancreatic cells. These high concentrations add to mentioned above were furthers contributes to over physiological insulin releasing cause obvious damage within pancreas' islets^[13-15]. This study was aimed to:

- 1- Pointed the physiological effects of D.M.
- 2- Reported the histopathological changes of different organs in mouse after induced diabetes mellitus.

MATERIALS & METHODS

Current experiment was conducted in 30-mixed white mice of uniform age (4week) at (18-24) gram body weight. They divided into sex groups of 5 animals to each (three groups were experimental and three groups were control); Group I was injected with alloxan for one week; Group II was treated for two weeks; Group III was for three weeks. Others three groups were controls besides each experiment groups and given normal saline or distilled water. Animals were housed in animals' house in Veterinary Medicine College of Baghdad University and given commercial-pellet feed, and tap water. Alloxan monohydrate (150 mg/kg) has been prepared in 10% saline solution and injected in mice intraperitoneal at single dose of (0.1ml/10 mg B.W) weekly for three weeks, Sugar level of these mice was test at 1-2-3 weeks for

diabetes detection on blood from each mouse blood by glucometer (ACCU-CHEK, Germany). Animals have more than 200 mg/dL were diabetic [1,16]. Tissue samples from different organs were fixed with formalin (10%) processing and prepared slides done according to [17].

Statistical analysis

Test data depend on means ± S.D. and analyzed according to ANOVA and t test. *P* < 0.05 considered significant (18).

RESULTS & DISCUSSION

Estimation of Glucose level

Each mouse of each two groups submitted to test for glucose using glucometer on samples of blood (ACCU-CHEK, Germany). As shown in table (1).

TABLE 1: showed results of mice blood glucose level, the estimation performed after alloxan injection as comparison to control mice

Animals groups	Time of treatment with Alloxan	Glucose level mg/dL(rang)
Group I / n=5	One week	80 -120 mg/DL***
Group II / n=5	Two week	125 -175 mg/dL**
Group III / n=5	Three week	200 -400 mg/dL*
Control / n= 15	Isotonic solution only	70 -100 mg/dL

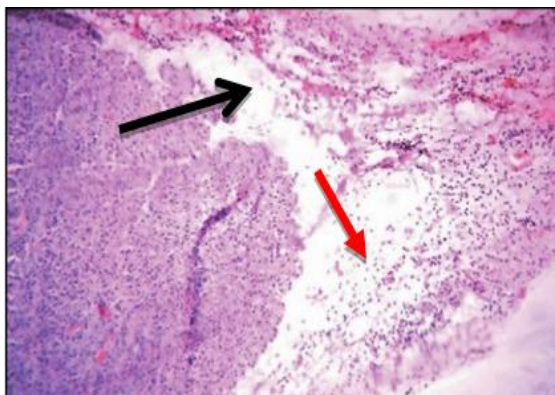
Significantly different from corresponding control at variance: **p*< 0.05;** *p* <0.01;*** *p* < 0.001

There was high glucose level after two week of alloxan injection with comparison to healthy mouse and there were significantly differences at *p*< 0.05 and these results are agree with [1,19]. Increase glucose level occurs due to failure of body function that's associated to glucoses' and lipids' metabolism [20].

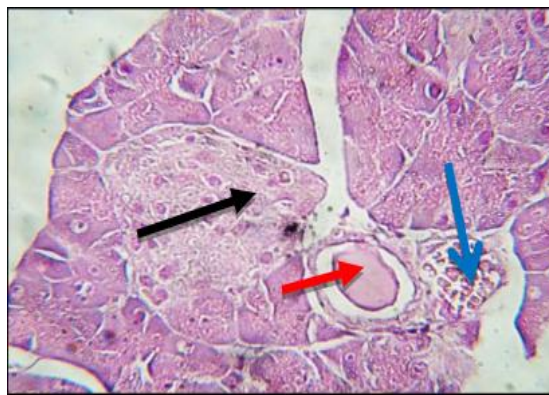
Histopathological Investigations

Pancreas

Histopathological investigations on pancreatic section showed; inflammatory infiltration and necrosis in interlobular septa destructive change on epithelial lining cell with hemorrhage and congested blood vessel. As showed in figure (1-A; B).



A



B

FIGURE 1 A: Pancreatic section of mouse showing cellular infiltration in interlobular septa (black arrow) with necrosis (red arrow). (H&E X10).

FIGURE B: Pancreatic section of mouse showing necrosis within lobules (black arrow) vessels dilation ' with thrombi (red arrow) with hemorrhagic changes (blue arrow).

Pathological lesions in pancreases were severe necrotic changes after two weeks; increased eosinophilia (nuclear fragmentation), cellular infiltration in interlobular septa, hemorrhage, fibrous' proliferating in pancreatic lobules and pancreatic' acini disarrangement's, nuclear changes: karyohexis and fragments' then absent of nucleus and rarefies in nuclei on cells [19]. And degeneration islets of Langerhans are induced by alloxan were agreed with other authors findings in animals [14, 21, 22].

Liver

Liver tissues showed degenerative hepatocytes with hepatosis with fatty droplet as clear vacuoles; infiltration of kuffer cell; cloudy swelling and absence of sinusoids; hemorrhagic change with degenerative changes within hepatocytes including absence of nuclei, Figure (2-A; B). Histopathological findings in liver were agreed with (23).

These liver changes are due to alloxan induced D.M [21; 24] in spite liver, insulin-dependence organ and play a vital role in Sugar and fat homoeostatic', is seriously affects' during diabetes[25]. Causes' prominent alteration in the lipids' concentrating and compositing[26]. Disturbance in sugar metabolizing so there was decreased glycolysis, stimuli glycogenesis and increased glucose-genesis in diabetics' liver[27]. Other authors said that untreated diabetics' liver cause hyperglycemia which is known to activate isoforms of proteins' kinases C (PKC) in other tissue[28] besides hepatocytes, PKC is major step for insulin transductions' pathway which stimulates PKC [29]. Leads to decreased apoptosis and hyperplasia that's termed diabetics' hepatomegaly [24].

Brain

Brain and cerebellum showed widely speared degenerative changes and these appear as necrotic foci and there are

multiple area of vacuolation of neuron due to demyelination (fatty degeneration), Figure (3).

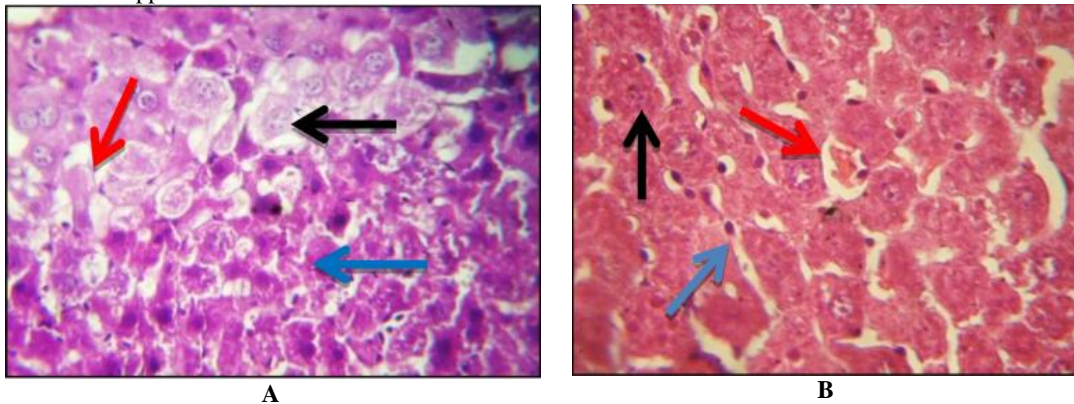


FIGURE 2A: Liver section of mouse showing hepatosis (black arrow) and fatty change (red arrow) and cloudy swelling (blue arrow) (H & E X 40).

B: Liver section of mouse showing hemorrhage (red arrow) with degenerative hepatocytes with fragmented nuclei (black arrow) with infiltration of kuffer cell (blue arrow). (H & E X40).

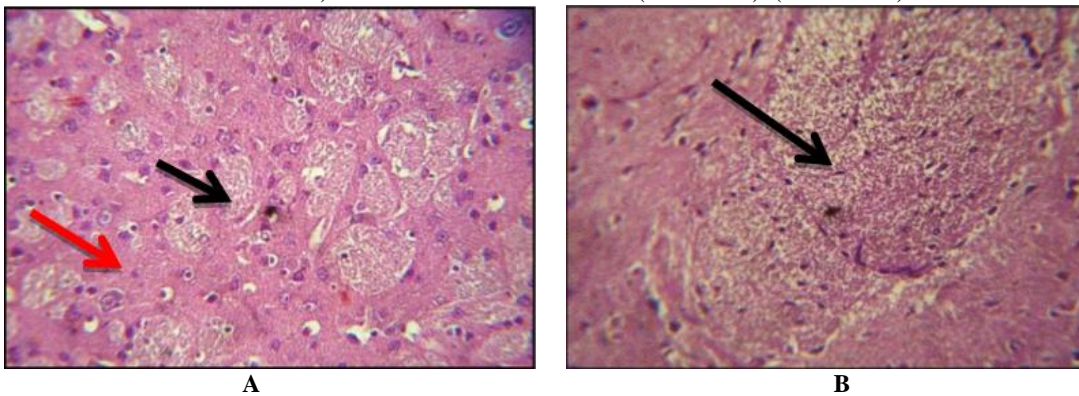


FIGURE 3A: Brain tissue of mouse showed: necrosis (black arrow) with neuronal vacuolation (red arrow) (H&E).X100.

B: Brain tissue of mouse showed: necrotic foci arrow X 400.

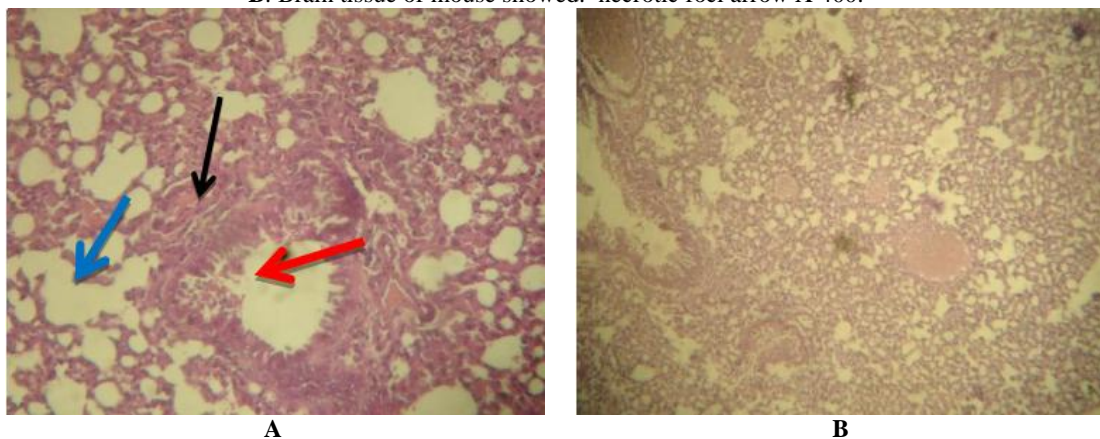


FIGURE 4: Lung sections of mouse: A showing congestion (black arrow) and desquamation epithelium of bronchioles (red arrow) with emphysema (destruction of alveoli wall) (blue arrow) (H&E) X 400.

B: showing Interstitial Pneumonia as whole slide (H&E) 100X.

These changes in nervous system after alloxan injection, termed diabetics' neuropathy and affects: brain; spinal cord and peripherals nerves [23, 30]. Diabetes initiates brain damage in experimental and clinical subjects, promotes neuronal damage; increases infractioned postischaemic seizures [31]. Diabetic neuropathy associated with excessive

production of sorbitol through reduction of maintaining hyperglycemia and related processes [32; 33].

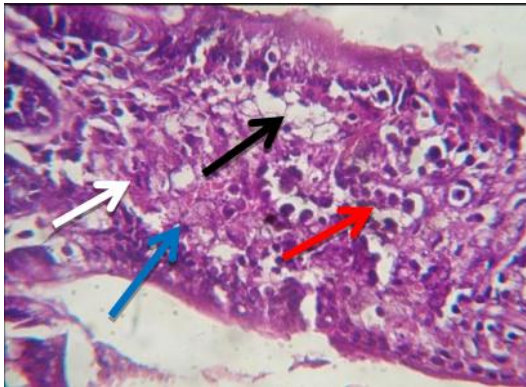
Lung

Our investigations on lung showed evidence of Pneumonia represented by inflammatory infiltration type with bronchial wall damage; alveolus wall damage with heavy cellular debris and exudation, Figure (4).

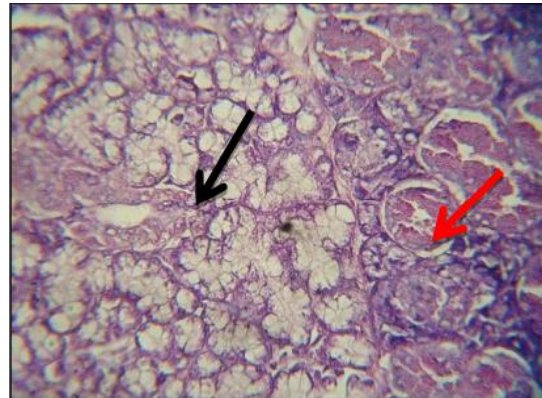
Changes in lung sections agreed with Author ^[21] who observed edema, collapse of alveoli, congestion and hemorrhage in lung sections of alloxan-induced diabetic dogs and agreed with author ^[34] in rabbit.

Gastrointestinal tract

Histopathological investigation on GIT and its glands showed fatty droplet with necrosis (Figure 5) and inflammation of Esophagus (Figure 6).



A



B

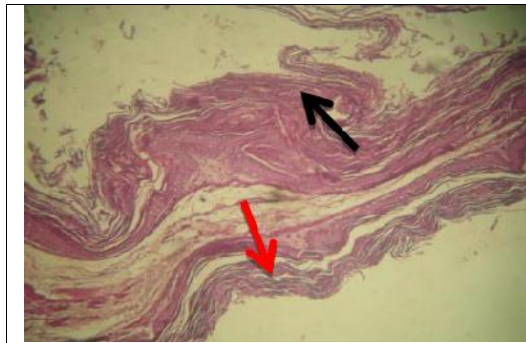
FIGURE 5A: G.I.T. section of mouse showing: marked fatty deposits (black arrow) with hydropic degeneration (red arrow) and necrosis (blue arrow) and mucinous degeneration (white arrow). (H & E) X100.

B: Retropharyngeal salivary gland with thymus gland section of mouse showing: congestion with necrosis in salivary gland (black arrow). AS well as in thymus gland (red arrow). (H & E X 400).

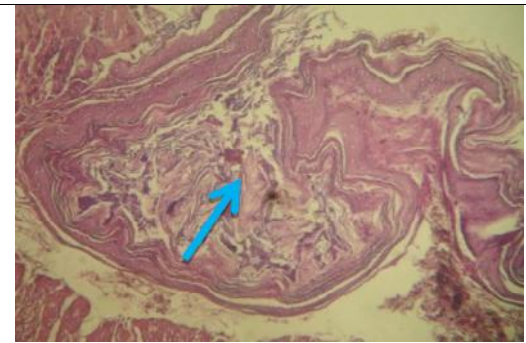
Esophagus

Histopathological investigation on esophagus showed different pathological changes characterized by hyperkeratosis with calcification in the wall of esophagus

accompanied with inflammatory responses mainly monocytes with fibrous connective tissues proliferation (Figure 6).



A



B

FIGURE 6A: Esophagus section of mouse showing: hyper keratinization (black arrow) with inflammation and thickening and increase proliferation of fibrous connective tissue F.C.T (red arrow). (H&E) X100.

B: Esophageal gland of mouse showed metaplasia with calcification and keratin debris (blue arrow) (H&E) X400.

These changes in G.I.T. resulted from disturbances in cellular homeostasis and vascular system in diabetes mellitus (35) no controlling stimulus of hypertrophy's and hyperplasia's and dysplasia and metaplasia of intestines' epithelium in diabetic animals are due to chronicity period accompanies with growth abnormalities ^[36]. Previous studies in experimental animals caused intestines' degeneration and necrosis on intestinal villi ^[21] and gained weight with high tissue waters' content ^[37]

increases growth of yeasts on the GIT mucosa also caused permanent hyperglycemia in diabetic rabbits. Hyperglycemia in D.M responsible for hurt tissues injuries ^[38].

Heart and Spleen

Pathologic investigations on heart and spleen showed inflammatory reaction and fat deposition with hemorrhage Figure (7).

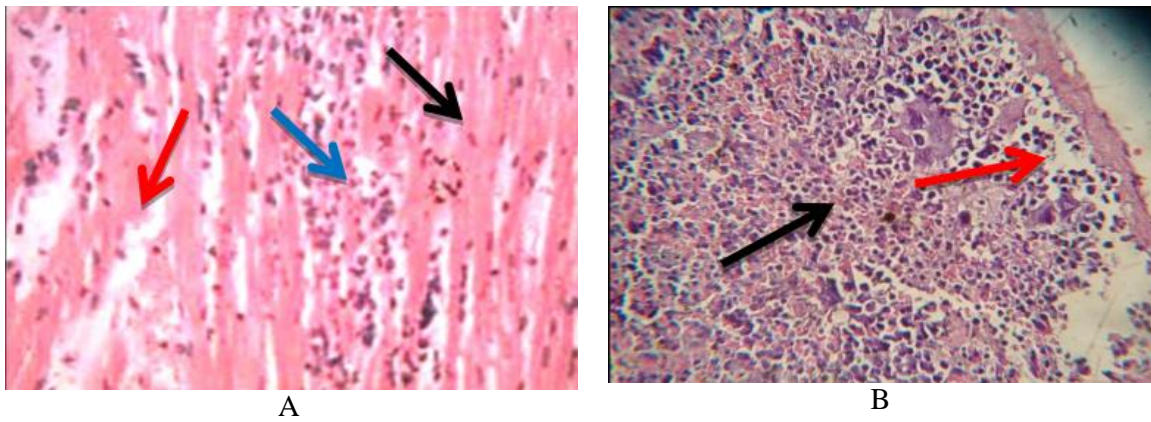


FIGURE 7A: Heart section of mouse showing congestion (black arrow); myofibrils hyalinization (red arrow) ; inflammatory cells (blue arrow) (H & E) X 400.
B: Spleen section of mouse showing dystrophic change and hemorrhage (black arrow) with evidence of fat deposition (red arrow) (H & E) X 400.

Changes in heart are agreed with author (34) who found edema, histiocyte proliferation and myocarditis in heart sections in diabetic rabbits.

Kidney

Sections of kidneys showed, Nephritis with hyaline and hemorrhagic cast with atrophied glomeruli and necrosis of renal tubules (Figure 8).

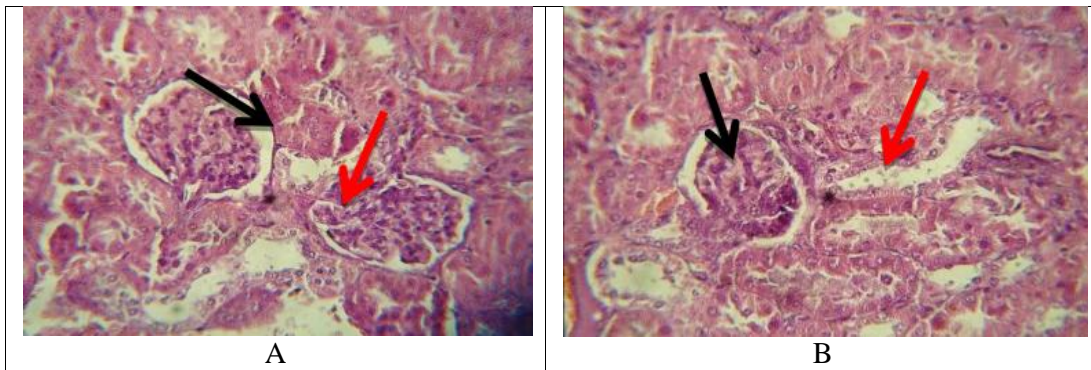


FIGURE 8: Kidney section showing: A: Nephritis with hyaline and hemorrhagic cast (black arrow)and glomeruli necrosis (red arrow).X400
B: Atrophic glomeruli (black arrow) with necrotic proximal tubules (red arrow). (H&E) X400.

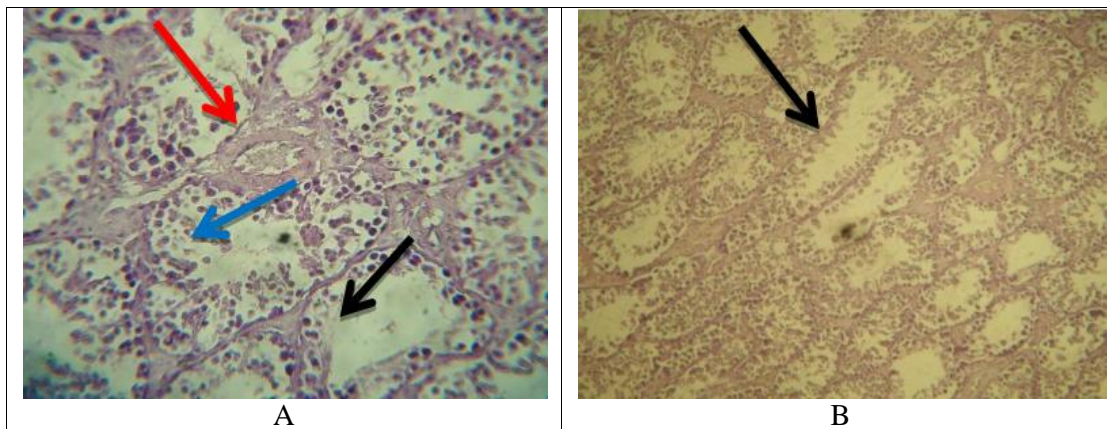


FIGURE 9: Testis section showing: A: empty seminiferous tubules from loss of spermatogenesis (black arrow) with congestion (red arrow) and fatty droplet within cell of epithelial lining.(blue arrow)(H & E X400).
B: empty seminiferous tubules from loss of spermatogenesis (arrow))(H & E X100).

Our changes in kidneys were agreed with author^[23] and they are attributed to defecated metabolism^[39] in hyperglycemic or diabetic animals^[22, 40] other authors showed proliferations' cell changes and nephrons hypertrophy in diabetics' rabbits. In diabetics' dogs there are degenerating glomeruli with necrotic tubules' epithelium existence of hyaline casts^[21] several researchers showed glomerular nephropathy and sever interstitial changes ^[39,41] and others showed that controlling metabolism is essential for reduction of nephropathy in diabetes, and when there is prolonged diabetes' duration and no normoglycaemia reinstitions' so nephropathy is difficult reverse to normal status ^[42, 43].

Testes

Tissues' of testes showed failure of spermatogenesis with inflammation represented by infiltration of MNCs enter and outer seminiferous' tubules with proliferation and thickening of fibrous connective tissue septa with evidences of congested bloods' vessels; necrosis within lumen of tubules.(Figure 9). These results on testicular tissue of mice after alloxan uses are agree with authors ^[44, 45].

Skin

Skin tissues after alloxan injected mouse showed loss of hair follicles with hyperplasia of collagen fibers and fibrous connective tissue F.C.T. (Figure 10).

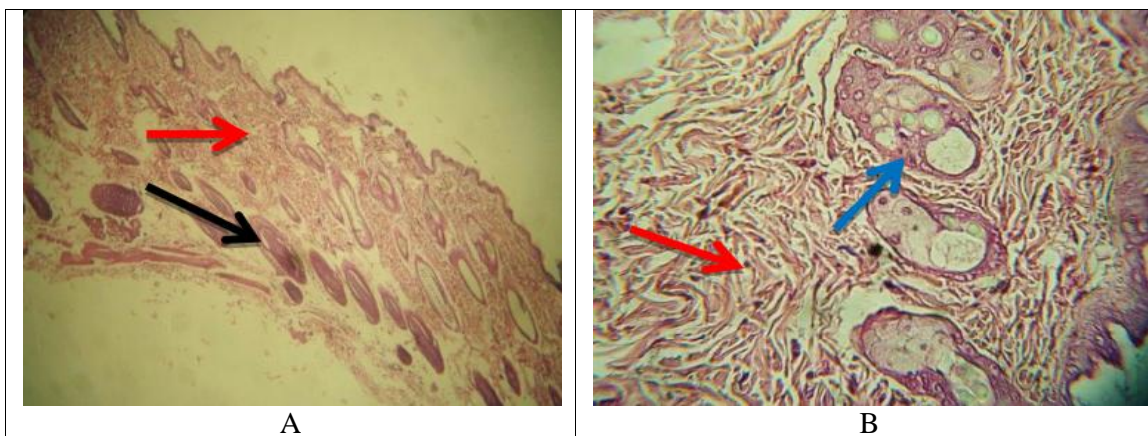


FIGURE 1: Skin section showing A: aborted hair follicles (black arrow) and inflammatory response (red arrow) increase F.C.T thickness. (H & E X100).
B: atrophied sebaceous and sweat gland (blue arrow); increase F.C.T thickness (red arrow) (H &E ×400).

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