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Investigation of Ultra Structural Changes in Testicles of German Shepherd Dogs Following Alloxan Induced Diabetes Mellitus

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Abstract: *Diabetes mellitus* is one of the most common diseases of endocrine glands that is diagnosed by malfunction in natural metabolism of carbohydrate, fat and protein. This disease involves most tissues of the body and the consequent deficiencies reduce their efficiency, cause infections and malfunction in different organs of the body. Alloxan is a chemical substance which is used in creating experimental diabetes in animals. In this investigation, 9 healthy German shepherd dogs were used, five of which were considered as the experimental group and the remaining four were considered as the control group. The necessary examinations were conducted to guarantee their health and they approved to be the absence of diabetes free with intravenous Glucose Tolerance Test (IVGTT). In this study, the experimental group was I intravenously injected with Alloxan monohydrate (100mg/kg). Induced diabetes was approved by IVGTT. From the experimental group, showing signs of the death and from the control group (after end of experiment) specimens were taken from testis and processed for microscopic and ultra structural examinations spermatogonia showed vacuolization of cytoplasm and cell degeneration. Spermatogenesis process was faced derangement in testicles and was stopped in one of them. It was concluded that Alloxan Induced Diabetes Mellitus, may cause detrimental effects on Spermatogenesis and testicle function in dogs.

Key words: Ultra structure · Alloxan · *Diabetes mellitus* · Testicles · Dog

INTRODUCTION

Diabetes mellitus is one of the endocrine related diseases in human and animals which involves the blood circulatory system. About 6.3% of world populations are living with diabetes. Diabetes creates the following common symptoms during its chronic length: thirst, poly urea, increased appetite and weight reduction, heart and coronary problems, kidney problems, sight problem, coma, shock, ketosis, increased blood glucose, increased blood pressure and soon [1].

The factors that contribute to diabetes may be as following: hesitation, pancreas deficiencies (infections, immune deficiency and tumors), drug and chemicals, environmental factors (obesity, stress, high blood pressure, physical inactivity, aging and high cholesterol) [2].

Due to formation of suitable condition for diabetes, the disorder may increased in animals. Dog is one of the animals which has the most diabetes case among animals. In the other hand, dog can be a useful laboratory model for studying of diabetic deficiencies and in this way help veterinary and medical researchers. In our pervious study deficiencies in dog kidney after alloxan induced *diabetes mellitus* was evident [3].

The aim of this study was to investiogate macroscopic, microscopic and ultra structural deficiencies of testis in German shepherd dogs following experimental diabetes via Alloxan.

MATERIALS AND METHOD

In this study, 9 male German shepherd dogs with age of 1.5-2 years old were used. These animals were apparently healthy as monitored by clinical examinations and survival signs control and had no special disease in their history. Dogs were transferred to research institute of Islamic Azad University-Shabestar branch. All animals were numbered and weighed. Next To make sure, they were given Antiparasitic *Levamisole*® in dose of 10mg/kg.

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Meanwhile, Rabies vaccination was injected under the supervision of local veterinary organization.

A 32meter square space to keep them in research center of university which is equipped with ventilation system was provided. Dogs were kept in Animal room, but they could easily move in a limited space, water and food were available according to their requirements. The numbering was from 575 to 583 and 5 of them were considered as the experimental group and the other 4 as the control group.

In order to make them adapted to the prevailing condition and to avoid stress, Dogs were not subjected to any experiment for one week but during this period, they were checked for clinical signs.

After adaptation period, IVGTT experiment was applied to make sure of the absence of diabetes. Then after 5 days, 100mg/kg of Alloxan monohydrate (Sigma®) was injected (IV). 3days later, one of the dogs in this group died and autopsy was done quickly. A week later, the second IVGTT was done and the presence of diabetes was approved.

During the whole time in the experiment, the dogs in both groups were examined carefully for clinical signs (anal temperature, heart rate, respiratory rate,...).

When animals in the experimental group indicated dangerous symptoms, they were studied rapidly. If the symptoms were indicating the death of the animal, to avoid any mortality of the animal during night and to remove autolysis, autopsy was done and took sample of the animal tissues.

The samples for light microscope were fixed in formalin buffer (10%) and the samples for electronic microscopy were fixed in glutar-aldehyde buffer (3%). After that, the next steps of tissue processing was done. After finishing the experiment autopsy was carried out and specimens were taken from testis of the control group. Electronic microscope sections were colored with standard methods using uranil acetate and lead citrate [4].

RESULTS

Clinical Symptoms: At the beginning of the disease which appeared about 30 hours after injection of Alloxan, due to diabetic acidosis, dogs had symptoms like lack of appetite, vomiting, thirst, poly urea, increased breathing, low consciousness, dehydration, slight hypovolemic shock signs and tachycardia. Body temperature slightly decreased.

Dogs had stomach ache due to acidosis, tiredness and lack of electrolyte balance. Sever hyperglycemia, increased plasma osmolarity and decreased body fluid which contributed to decrease of consciousness and coma.

Alopecia was observed in a single case (case number 581). Survival parameters are shown in Tables 1 and 2, changes in dog's weight are shown in Table 3, duration of living of dogs after Alloxan injection is shown in Table 4 and comparison of water consumption mean to dog's weight in different times is shown in Table 5.

Table 1: Survival parameters in the start of test in all of dogs

	Control				Experimental				
	No.								
Group									
Parameters	578	579	580	583	575	576	577	581	582
Temperature (°C)	38.6	38.7	38.3	38.5	38.7	38.5	38.4	38.2	38.7
Heart rate (per minute)	97	93	87	89	94	92	85	89	82
Respiratory rate (per minute)	30	28	29	29	32	30	35	28	26

Table 2: Survival parameters in the end of test, control and experimental groups of dogs (after injection of alloxan to experimental groups)

	Control				Experimental				
	No.								
Group									
Parameters	578	579	580	583	575	576	577	581	582
Temperature (°C)	38.5	38.3	38.1	38.6	38.9	37.7	38	38.4	37.8
Heart rate (per minute)	94	95	90	86	117	119	121	124	130
Respiratory rate (per minute)	32	31	30	28	39	38	40	37	38

Table 3: Changes of weight in control and experimental groups of dogs in duration of research (Kg)

	Control				Experimental				
	No.								
Group									
Time	578	579	580	583	575	576	577	581	582
First IVGTT	26.5	24	38.3	25.3	29.5	31	26	27.2	23
Injection of Alloxan in experimental group	27	24.6	38.5	26	30	32	27	28	23.5
Second IVGTT	27.5	25	39	27	29	30	-	26.5	22
End of Test	28.2	26	40	28.1	25	27	24	23.1	21.5

Table 4: Duration of survival in experimental groups of dogs after injection of alloxan

No.	577	582	576	575	581
Duration(day)	3	14	18	38	43

Table 5: Comparison of water consumption mean to dog's weight in different times

	Control	Experimental
	Parameter	
Group		
Time	water consumption mean to weight	water consumption mean to weight
After IVGTT-1	0.0745	0.759
After IVGTT-2	0.0757	0.120

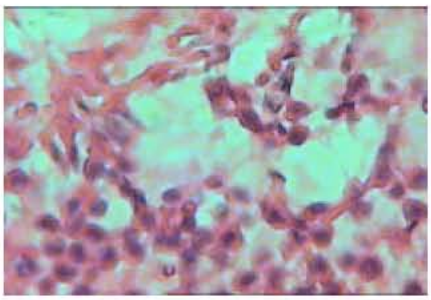

Fig. 1: Testis in the control group of dog, revealing healthy spermatocytes and spermatozoids '(X400, HandE)

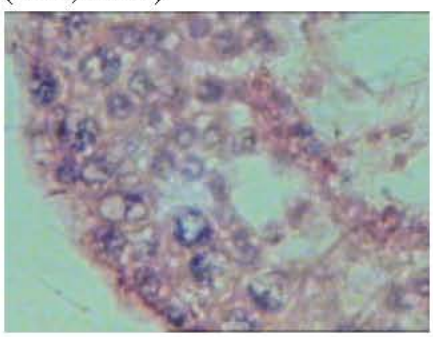

Fig. 2: Testis in dog after injection of Alloxan and induction of *diabetes mellitus revealing,* some vacuoles in spermatocytes. Spermatogenesis process was stopped. Degeneration process was seen in spermatocytes. Some of them show cell necrosis (X400, HandE)

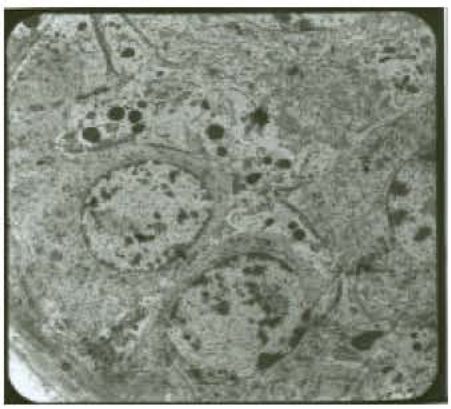

Fig. 3: Electron micrograph of spermatogonia cells in healthy dog (X4950)

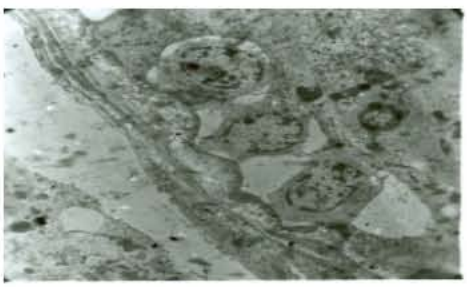

Fig. 4: Electron micrograph of spermatogonia cells after injection of Alloxan and induction of *diabetes mellitus* showing vacuolization of cytoplasm, cell degeneration, big hallow space between spermatogoni cell (X3596).

Microscopic Deficiencies: In the control group, healthy spermatocytes and spermatozoids. (Fig. 1) were obvious. In case number 575,576 (experimental group) some vacuoles were seen in spermatocytes. Spermatogenesis process was stopped. Degeneration process was seen in spermatocytes. Some of them revealed cell necrosis. The number of Leydig cells decreased (Fig. 2).

Electronic microscope deficiencies: In the control group, healthy spermatogonia cells were seen (Fig. 3) In the experimental group, vacuolization, decreased in size of cytoplasm and cell degeneration were seen. There was big hallowing space between spermatogonia cells. Basal membrane showed thickening (Fig. 4).

DISCUSSION

The incidence of diabetes is reported one in 200 [5]. Incidence of *diabetes mellitus* by itself in dog either elementary or secondary is due to pancreas atrophy because of beating, by itself atrophy, pancreas hypoplasia, aplasia, dysplasia, sexual cycle (estrous stage) pancreatitis, influence of amyloid,glycogen, collagen and connective tissue in langerhans islets, treatment with prednisolone, acidophil cell's adenoma in hypophysis, obesity, infection and hyperadrenocorticism together with pregnancy diabetes [6].

Diabetes among dogs is usually seen in middle age and old dogs. In order to create experimental diabetes in animals, pancreatectomy or prescribe chemical drugs such as Alloxan, streptozotocine and so on can be used [7].

The normal dose of creating diabetes in dog with Alloxan is 65-200mg/kg in the form of IV [2]. The injection speed is very effective and the drug should be provided recently [8].

Clinical signs in humans and animals are almost the same which include: over thirst, Severe thinness, increase of urine, increase of appetite, hyperglycemia, glycosuria and ketonuria. In this investigations, the above mentioned symptoms were also seen. Also,we observed abnormality in respiratory system, loss of hair and diarrhea [9].

CONCLUSION

In microscopic study of experimental group, vacuoles were seen in spermatocytes. Spermatogenesis process was stopped. Degeneration process was seen in spermatoctes and some of them show *diabetes mellitus* were necrosis. In ultra structural study, in the spermatogonia cell of testis, showed vacuolization of cytoplasm, decreased size and cell degeneration. Basal membrane is thickened. There is big hallowed space between spermatogoni cells.

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