

EFFECT OF L-ARGININE ON SPERMATOGENESIS OF THE DIABETIC RAT

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ABSTRACT

L-Arginine has hypoglycemic and antioxidant effect in Alloxan diabetic animals and reduce effect of diabetes complication on spermatogenesis. antioxidant have essential effect on spermatogenesis, L-Arginine has antioxidant and hypoglycemic effect. Enhanced oxidative stress and changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus.

Sixteen mature male rats aged 10 weeks ,were randomly divided into four equal groups as follows 1st diabetic group (DG) that received 150 mg/kg (IP) Alloxan single dose ;2nd group diabetic group treated with (L-Arginine-Hcl)(DAG) received 150mg/kg (IP) Alloxan as single dose plus L-Arginine-Hcl 200mg/kg(IP)-per day, 3^{ed} group treated with(L-Arginine-Hcl)(AG) received 200mg/kg-(IP) perday ,and 4th were control group(CG) non treated .In 60 day the blood samples collected from heart to make serological parameters (glucose level) and testes removed to observe their Histopathological.

Serum Glucose concentration showed a significant increase ($p<0.05$) in diabetic group (DG) compared with other groups while diabetic treatment group that has received L-Arginine 200 mg/kg(IP) (DAG) showed a significant ($p<0.05$) decrease compared with (DG).

The testes statistical analysis showed significant decrease ($p<0.05$) in testes weight in diabetic group compared with other groups.

No histopathological changes was observed in both control and Arginine groups (CG &AG),meanwhile the histopathological change in testes of diabetic group (DG) showed Epididymal tubules showed completely empty, The DAG showed Epididymal tubules of DAG group showed little spermatozoa.

Since in our study 200 mg/kg (IP) L-Arginine have significantly Preventive effect on serum glucose level and spermatogenesis, so it seems that using it can be effective for treatment in Diabetic Rat.

INTRODUCTION

Diabetes mellitus is a chronic metabolic disorder characterized by a high blood glucose concentration due to insulin deficiency and/or insulin resistance (1), also altered metabolism of lipids, carbo-hydrates and proteins, and an increased risk of complications(2), enhanced oxidative stress with changes in antioxidant capacity are considered to play an important role in the pathogenesis of chronic diabetes mellitus (3), the generation of free radicals often worsen the complications of diabetes mellitus such as hypertension, atherosclerosis and microcirculatory disorders (4,5). The balance of free radicals and antioxidant is a major mechanism in preventing damage by oxidative stress. Therefore, the dietary supplement of antioxidants such as vitamins, flavonoids has been used to prevent the occurrence of complication in many chronic diseases like diabetes mellitus (6).L -Arginine is a semi-essential amino acid in most mammals and can increases insulin secretion (7), also recently reported beneficial effects of L-Arginine supplementation on reducing serum concentrations of glucose and lipids in diabetic rats (8). L-Arginine reduces vascular oxidative stress and inhibits superoxide generation (9,10). The male reproductive function is clearly impaired in diabetes. Diabetes-induced alterations of Leydig cell functions include a decrease in androgen synthesis and in the total number of these cells (11). Also diabetic men have lower testosterone levels compared to men without a history of diabetes and that lead to defect in spermatogenesis (12,13) it is hypothesized that L-

Arginine can decreasing harmful effect of diabetes on spermatogenesis by reducing or normalize blood glucoses level and reactive oxygen species.

MATERIALS AND METHODS

Animals of experiment:

A total number of 16 albino mature male rats were used in this investigation. The rats were 10 weeks old, body weight (125g-280g with range195g). Animals in all stage of the experimental were maintained under uniform environmental conditions, The rats were kept at a temperature between 21-28 C° and kept in plastic cages (56,40,17 cm), The light and dark cycle was (12:12hr). Rat had free access to fed ordinary pellet diet and water. The animals were adapted for 2 weeks and allocated randomly.

Experimental set up

sixteen of albino male rats were divided into 4 groups.

1-Control group (CG).Consists of 4 animals. They were receiving daily for 60 days a single dose of sterile distal water intraperitonealy.

2- L-Arginine-HCl control group (AG).This group consists of 4 animals. They were receiving daily a single dose of L-Arginine-HCl intraperitonealy (200mg/Kg B.W) for 60 days.

3-Diabetics group (DG). This group consist of 4 animals were receiving single dose of Alloxan monohydrate (150mg/kg) to induce diabetic. After (5days) they were received daily sterile distal water (0.5 cc /animal) intraperitonealy for 60 days after indication of diabetic.

4-Diabetics with L-Arginine-Hcl group (DAG): This group consists of 4 animals. They received single dose of Alloxan monohydrate (150mg/kg) to induce diabetic ,after (5days) they were received daily single dose of L-Arginine-Hcl intraperitonealy(200mg/Kg B.W) for 60 days.

Induction of experimental diabetes

Diabetes was induced by a single is injection of alloxan monohydrate(Sigma Chemical Co, United State of America),((150 mg/kg dissolved in sterile normal saline)) after fasting the rats for 12 hours (14) After 72 hours of alloxan injection, the diabetic rats (glucose level > 135 mg/dl) were separated and used for the study as diabetic rats .

L-Arginine preparation

L-Arginine was obtained from BDH Chemical Company (ENGLAND). Prepared immediately before use by dissolving (1 gm) of L-Arginine Hcl in 10 ml sterile distilled water (10%).

Blood glucose determination

Blood samples were collected at end of experiment at 60 days of via cardiac puncture from each anaesthetized rat (Ketamin hydrochloride with Xylazin) after fasting 8-12 hours, using disposable syringes. Samples were centrifuged at 3500 rpm for 15minutes then serum glucose measured using enzymatic colorimetric methods according to the BioLinear chemicals kits (SPAIN).

Testes weight calculate

After surgical removal of testes its measured by Electronic balance (Shimadzu AY220 , Japan) .

Histopathological examination:

Specimens were immediately fixed in 10% formalin, the fixed testis tissue samples were dehydration by passing the tissue blocks through ascending grades of ethanol (70%, 80%, 90% and 100%) (1st run) and 100% (2nd run) then clearing by passing the tissue through xylene then embedded in paraffin, mounted on glass slides and stained with haematoxylin-eosin(15).

Statistical analysis

The statistical analysis was done by using the SAS system v. 6.11. Results are expressed as means \pm SE. Differences between groups were analyzed by one-way ANOVA, and if significant paired *t*-test or also called (LSD) least significant differences was used between individual data points. *P*-values are two-sided and considered significant when $P < 0.05$ (16).

RESULTS

The statistical analysis for Serum Glucose concentration (mg/dl) revealed that the DG showed a significant increase ($P < 0.05$) in serum glucose concentration (188.75 ± 11.24 mg/dl) compared with CG in the same period (116.00 ± 3.53 mg/dl).

Meanwhile DAG was showed significant ($P < 0.05$) decreased in serum glucose concentration at (112.00 ± 2.88 mg/dl) compared with DG in the same period. As shown in table (1).

The testes body weight results showed, the DG showed a significant ($P < 0.05$) decrease (1.21 ± 0.39) compared with other groups in same period. As shown in table (1).

The Histopathological study of both AG and CG rats' testes not showed architectural changes in the histology of at end of experiment (Fig 1, 2 and 3). While the DG Epididymal tubules showed completely empty tubules because of marked suppression in the spermatogenesis, The DAG showed Epididymal tubules of DAG group showed little spermatozoa.

DISCUSSION

Our study show that The DG showed significant increase ($P < 0.05$) in concentration of serum glucose concentration compared with CG in the same period that occur due to destroy of the β -cells in pancreas by Alloxan and absent of insulin secretion (17).also results demonstrate that administration of L-Arginine to the DAG showed significant ($P < 0.05$) decrease in the serum glucose concentration compared with DG in the same period. This may due to the following cause: The L-Arginine

stimulates glucose uptake and utilization by skeletal muscle by increasing GLUT-4 translocation to the plasma membrane (18).Increased in insulin secretion by pancreatic β -cells that are not destroyed after Alloxan (19).

The testes body weight was significant decrease also the completely empty tubules due to suppression in the spermatogenesis in DG may be result of hyperglycemia , Testicular atrophy and hypogonadism have been reported in diabetic men by (20), also diabetes induces a decrease in the serum levels of luteinizing hormone (LH), which is responsible for normal Leydig cell function (21,22).and that which lead to low testosterone levels (Zietz B, 2000,Agbaje et al, 2008) The Diabetic testicular dysfunction might be transient or permanent depending on the degree and duration of the disease. Erectile dysfunction (ED) is a well recognized complication of diabetes mellitus (DM). The low incidence of diabetes in infertile patients might be the reason for the limited amount of current research (23). However, an altered testicular axis was noted in experimental studies by (24,25). found that testicular weight, sperm count and motility significantly decreased in diabetic rats. Moreover, (26,27). Together, these hyperglycemia causes increased oxidative stress because the production of several reducing sugars is enhanced via glycolysis and the polyol pathway (28).Oxidative stress plays a role in the development of diabetic complications (29). research that showed testicular injury and apoptosis induced by diabetes are partially attributed to the augmented oxidative stress in testicular tissue. Previous studies indicated that reactive oxygen substances may be involved in possible testicular complications in diabetic of rats (30).the DAG showed normal testes body weight and showed little spermatozoa may due to that L-Arginine normalize hyperglycemia and can act us anti oxidant by scavenge superoxide anion $[O^{\bar{2}}]$ radical causing inhibition of oxidation process (31,32).

Table (1).

| DAYS GROUPS | Body weight | Testes body weight |
|----------------|-------------------|--------------------------|
| DG | 188.75±11.24 A | 1.21±0.39 B |
| DAG | 112.00±2.88 B | 1.65±0.40 A |
| AG | 120.00±3.57 B | 1.58±0.37 A |
| CG | 116.00±3.53 B | 1.62±0.51 A |

Values are expressed as mean ± SE

-n= 4/group

-The letters denote significant difference (P<0.05) within a column.

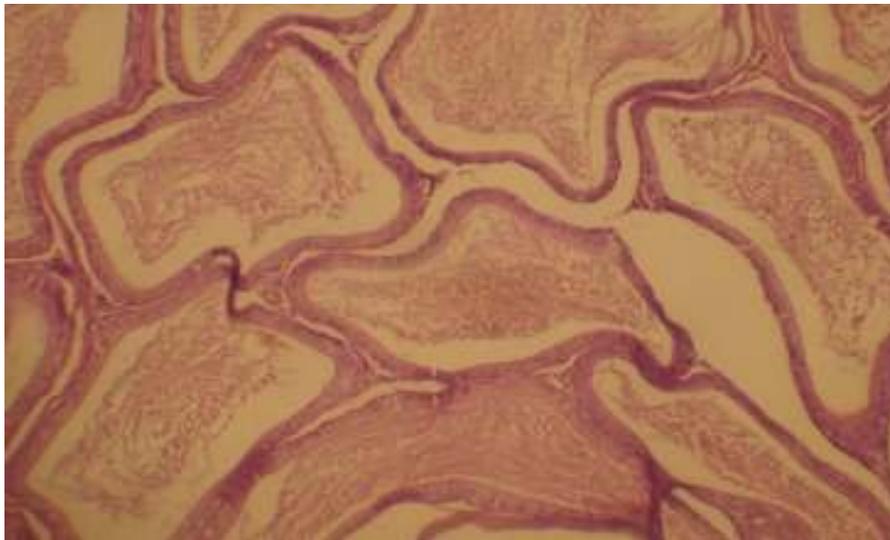


Fig (1) epididymal tubules of CG group showed presence of spermatozoa in the epididymal tubules. (100 X H&E)

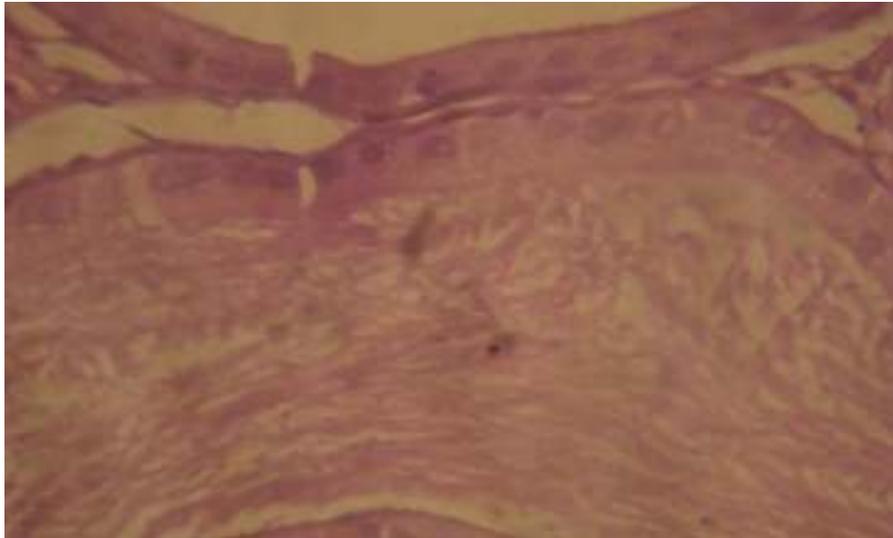


Fig (2) Epididymal tubules of CG group showed presence of spermatozoa in the epididymal tubules. (400 X H&E)

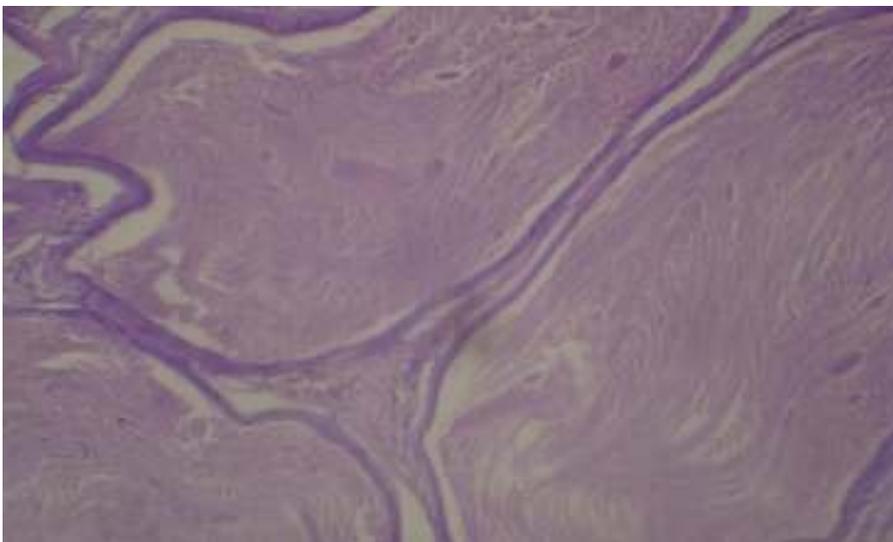


Fig (3) Epididymal tubules of AG group showed marked spermatozoa in the Epididymal tubules.(400 X PAS)

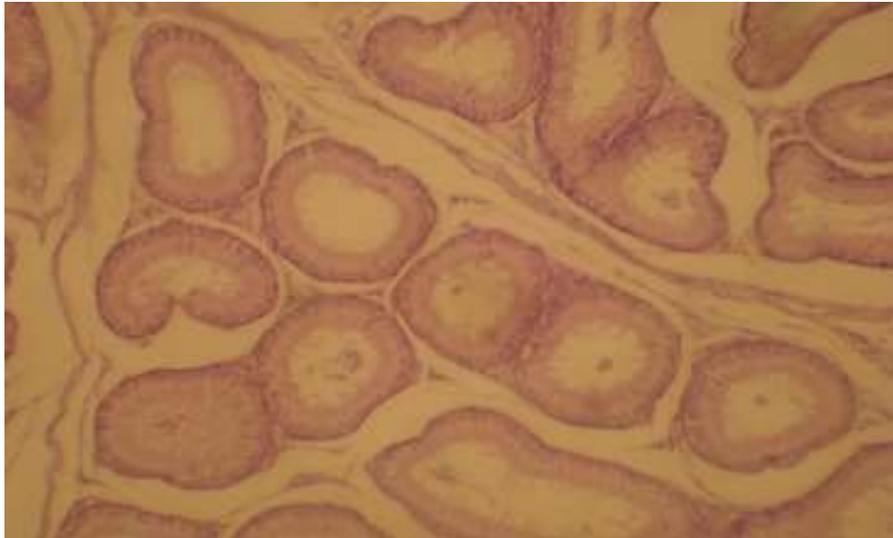


Fig (4) Epididymal tubules of DG group showed completely empty tubules because of marked suppression in the spermatogenesis (100 X H&E)

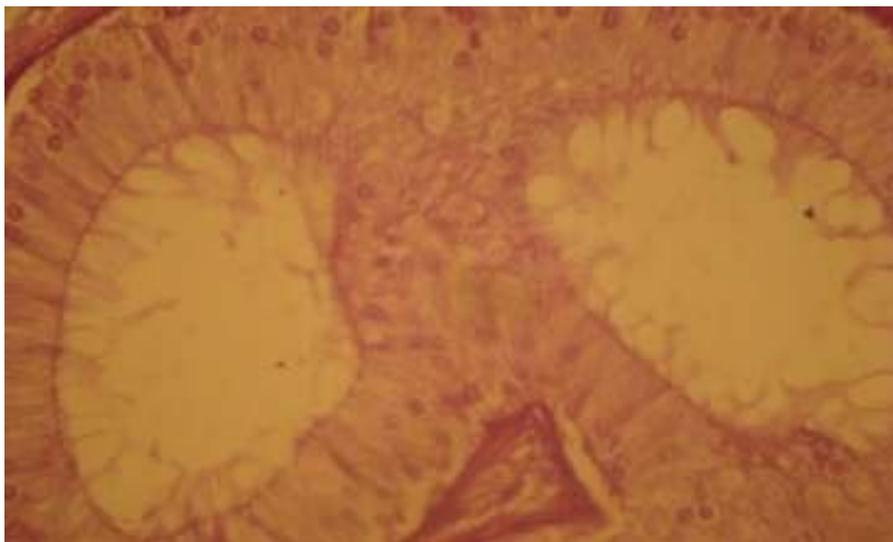


Fig (5) Epididymal tubules of DG group showed completely empty tubules because of marked suppression in the spermatogenesis (400 X H&E)

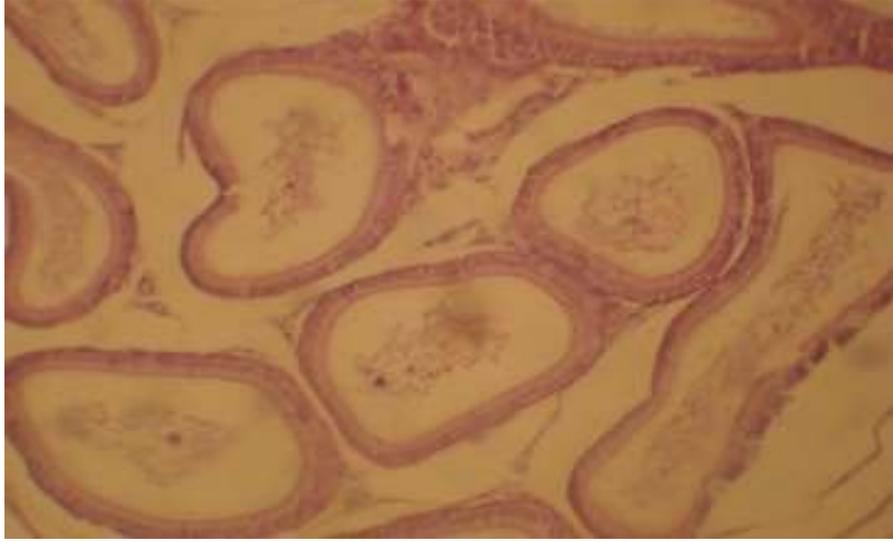


Fig (6) Epididymal tubules of DAG group showed little spermatozoa. (100 X H&E)

تأثير (ال-ارجينين) على عملية تكوين النطف في الجرذ البالغ المصاب بداء السكري

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الخلاصة

يعتبر الحامض الاميني (ال- ارجينين) من الحوامض الامينية التي لها فعالية مخفضة للسكر ويعمل كمضاد للجذور الحرة المتزايدة نتيجة لداء السكري والتي تعتبر السبب الرئيسي للمضاعفات المصاحبة لهذه المتلازمة ومن هذه المضاعفات الخلل الحاصل في عملية انتاج النطف. استخدمت في هذه التجربة ستة عشر جرذا من الذكور البالغة الخالية من الامراض ويعمر 10 اسبوع , والتي قسمت عشوائيا الى اربعة مجاميع تتكون كل (والتي اعطيت جرعة مفردة من الالوكسان وجرعة DG مجموعة من اربعة ذكور بالغه.(المجموعه الاولى)والتي جرعة بلالوكسان بجرعة 150ملغم\كغم ثم DAG 150ملغم\كغم حقنا في غشاء الخلب,(المجموعه الثانيه اعطيت الحامض الاميني (ال- ارجينين) بجرعة 200 ملغم\كغم في غشاء الخلب بشكل يومي لمدة 60 يوم (والتي جرعة (ال- ارجينين) فقط بجرعه 200ملغم\كغم في غشاء الخلب بشكل يومي اما AG , (المجموعه الثالثه المجموعه الرابعه هي مجموعته السيطره بعد 60 يوم من التجريع جمعت نماذج الدم من القلب مباشرة لغرض معرفة مستوى السكر في الدم في كل المجاميع وكذلك تم ازالة الخصى لغرض معرفة التغيرات النسيجية الحاصلة في كل مجاميع التجربة.

اظهرت النتائج ان هناك زيادة معنوية حاصلة في مستوى السكر في المجموعة الاولى والتي اعطيت الوكسان فقط مقارنة مع المجموعات الاخرى, اما نتائج المجموعة الثانية فوجد انخفاض معنوي في مستوى السكر في الدم بالمقارنة مع المجموعة الاولى. كما اظهرت النتائج نقص معنوي حاصل في وزن الخصى في المجموعة الاولى بالمقارنة مع المجموعات الاخرى, اما التغيرات النسجية للخصى والبربخ فوجد ان المجموعة الاولى المصابة بالداء السكري لوحظ عدم احتواء البربخ على النطف الناضجة بالمقارنة مع المجموعة المعالجة والمجموعات الاخرى اما المجموعة المصابة بالسكري والمعالجة فقد لوحظ احتواء البربخ على النطف.

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