

Determination of GLUT3 and GLUT8 Protein in the Testis of Diabetic Rats Treated with Aqueous Extract of Cymbopogon* Citrates

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Abstract

The present study was designed to obtain the localization of (glucose transporter 3) GLUT3 and GLUT8 protein in the testis of diabetic rats which were treated by aqueous extract of Cymbopogon citratus (100 mg/kg) once daily. Diabetes induced to the laboratory animals (Sprague dawly rats) by alloxan injection (90 mg / kg .bw. by a single intraperitoneal injection). The experiment lasted for five months during which the animals were divided into diabetic and non-diabetic normal groups. Significant increase was obtained in the localization of (glucose transporter 3) GLUT3 protein in diabetic testis in all age groups, with significant decrease in both (glucose transporter 8) GLUT8 protein localization in testes in all age groups in comparisons with normal groups.

In brief the herb used in this study resulted in the increase in GLUT8 protein (glucose transporter 8) with decrease in GLUT3 protein (glucose transporter 3), therefore one can conclude that the herb might be used for the minimized the disorder of male reproductive during diabetes.

Introduction

Experimentally, alloxan can induce diabetes by damaging the insulin producing beta cells of the pancreas, through liberation of H₂O₂ affecting the DNA strand [1]. Glucose is the major substrate for energy production in mammalian tissues; it is transported into the cell via facilitative glucose transporters (GLUT). The GLUT family consists of 14 members, which differ in their tissue distribution and substrate specificity. Expression of several GLUTs is controlled by hormones and environmental factors and differential expression is involved in various disease states such as diabetes and cancer [2,3, 4,5,6].

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A family of glucose transporters (GLUT) mediates the cellular uptake of glucose at the plasma membrane by facilitated diffusion, a very intensive positive immunoreaction for GLUT3 was found in sertoli cells, peritubular myoid cells, macrophage-like interstitial cells, testicular endothelial cells and early spermatocytes, spermatids , leydig cells , brain , circulating white blood cells and carcinoma cell line suggesting that it may be important in providing glucose to region of high metabolic activity [7,8,9,10].

Vannucci *et al* , (1997) [11] showed that the GLUT3 protein expression increased in the thalamus of the five week –old diabetic animals , on the other hand Kaneto *et al*. (1996) [12] found that GLUT3 protein increased significantly after exposure to high level of glucose , the same results obtained by Boileau *et al* , (1995) [13] found that this protein increased in pregnant diabetic rat placenta concluded that GLUT3 stimulated under hyperglycaemic condition so that this protein appear highly sensitive to glucose level and may be play a important function in the alteration of tissue function the same result obtained by (Angulo *et al* , 1998 ; Galardo *et al* , 2008) [14,15].

Lemongrass (*Cymbopogon citratus*) is a flavonoid herb an aromatic tropical with clumped bulbous stems that ultimating become leaf blades, it has a branched cluster of stalked flowers and that can grow in clumps to a height of 5 ft. The fresh stalks and leaves have green color and lemon like odor (Figure1). Lemon grass has antioxidant effect by inhibiting the elevation of serum level of glucose [16], preventing the increase in cholesterol. The main component in this herb is citral and beta-carotene which has an antioxidant effect [18].

Ojo *et al* (2006) [16] found that lemon grass has antioxidant effect by inhibiting the elevation of serum levels of malondialdehyde and catalase , explain that this plant able to prevent alteration to memebrane lipids by preventing the increase in cholesterol / phospholipids ratio.

When taken internally in recommended dosage lemongrass is not associated with any significant side effects, administered herb orally to adult rat for 2 months in doses up to 20 times larger than the estimated corresponding human dosage, this herb did not induce any effect which could be taken as evidence of toxicity [17].



Figure (1) : *Cymbopogon citratus* [18].

The aim of the study:

1. To determine the immunohistochemical changes (GLUT3 and GLUT 8 protein) in testes of alloxan –induced diabetic rat .
2. a trial to repair the diabetic changes in the testes by treatment with aqueous extract of *Cymbopogon citratus*.

Materials and Methods

One hundred and twenty six healthy adult male Sprague dawely rats weighting (200-250) gm, / College of Medicine /Haweler Medical in the animal house were housed in a well ventilated room ,at)University. The animals were exposed to alternat cycle of (12h light ,12h dark photoperiod (22±2C°). They were kept under observation for about two weeks before the initiation of the experiment [19] fasted overnight, their blood sugar was measured by taking a drop of blood from tip of the tail. The blood glucose was determined in mg/dl.by using on electronic glucometer (Accu – check Roche Diagnostic GmbH, Mannheim, Germany).. Diabetes was induced by a single intraperitoneal injection of alloxan monohydrate (BDH chemical Ltd. England) in a dose of (90 mg /kg) which obtained by previous pilot study , dissolved in normal saline immediately prepared before usage .The control animals received normal saline only [20]. Blood glucose was measured *after three days of* alloxan injection, Rat with fasting blood glucose level above 150 mg/dl were considered to be diabetic [21].

The animals were divided into three groups, six animals in each then were sacrificed after (1, 3 and 5) months from the start up of the experiments by chloroform inhalation. The testes, seminal vesicle and prostate were removed immediately from the sacrificed animals, fixed in bouins fixative for histological studies.

The required amount of fresh leaves of *Cymbopogon citratus* obtained from Tikrit city , cleaned and dried and extracted [22].

A pilot study was done in order to indicate the suitable dose of *Cymbopogon citratus* water extract required to reduce the high blood glucose [23].

The total of 72 male rats were used to study the immunohistochemistry of testes, they were grouped into two main groups;

Group (N) = 18 rats , not diabetic given normal saline once /daily, Group (D) 18 diabetic rats given normal saline once /daily, Group (N.CC) = 18 non diabetic rats given aqueous extract of Cymbopogon citrates each of 18 rats, Group (D CC) diabetic group given aqueous extract of Cymbopogon citrates ;100 m.g /k.g ; orally once daily, of 18 rats. Each main group subdivided into three sub groups each of six rats. Animals sacrificed at the following interval 1, 3, and 5 month from the beginning of the experimentation at rate of six animals in each step, as follows:

The testis samples taken from the sacrificed animals were fixed in bouins fluid, dehydrated in, cleared , embedded in paraffin wax, cut at (5) microns by rotary microtome (Leitz 1512, Germany 46194), all the sections were stained by haematoxylin and eosin and Immunohistochemical staining (Dako Cytomation. En Vision +Dual Link System-HRP (AEC) and (DAB), Code K4004 and k4005) (Polyclonal Rabbit antibody to glucose transporters 3[GLUT3] protein .Cat.NO.1469101876.Lot, no.284719.Quartett, Schichauweq. Berlin, Germany) [24].

Positive expression of GLUT3 in the cell membrane and cytoplasm and testosterone in cytoplasm gives brown colour with DAB and orange colour with AEC. The cells were calculated by the special computerised method called grid cell count .Positive cells were determined by counting 1000 cells. All significantly stained cells were considered positive and divided by 10 to acquire the percentage (immunostaining index), at least 10 HPFs were measured for each case for the purpose of scoring.

The extent of GLUT3, and immunostaining was assessed as: Negative: when index was <6%, Weak positive: when index was 7-15%, Strong positive: when index was 31-50%

GLUT3, score of 31-50% was regarded as over-expression of these [25].

Statistical analysis of the results:

Statistical analysis was done by using: (ANOVA) Analysis of Variance one and two way, 2. L.S.D. (less significant difference) for multiple comparison between groups and bar-chart, Statistical package for social sciences (SPSS) version 14 computer software, Statgraphics centurion X V and SAS. If (P-value <=0.05) identify there is a significant difference between groups.

Results

a. GLUT 3 protein: The data indicate that a significant increase in the activity of GLUT3 protein in the testes of diabetic rats at ($p \leq 0.05$) in all age groups with mean values (17.5) and (49.52) compared with non-diabetic animals with mean values (2.38) and (5.13) (figures 2,3).

b. GLUT 8 protein: A marked decrease in GLUT8 protein expression in the testes of diabetic rats at ($p < 0.05$) in all age groups with mean values (5.67) and (3.6) , in such away that the activity of this protein declined with the progression of the disease compared with normal animals are significant with mean values (7.7) and (14.97) (figure 3).

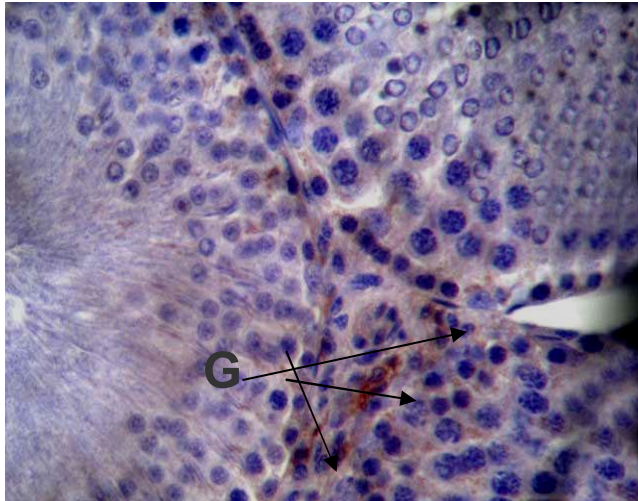


Figure 2: Section from normal rat testes after five months of experimental duration showing the amount of GLUT3 protein (G) (IHC ,X1000)

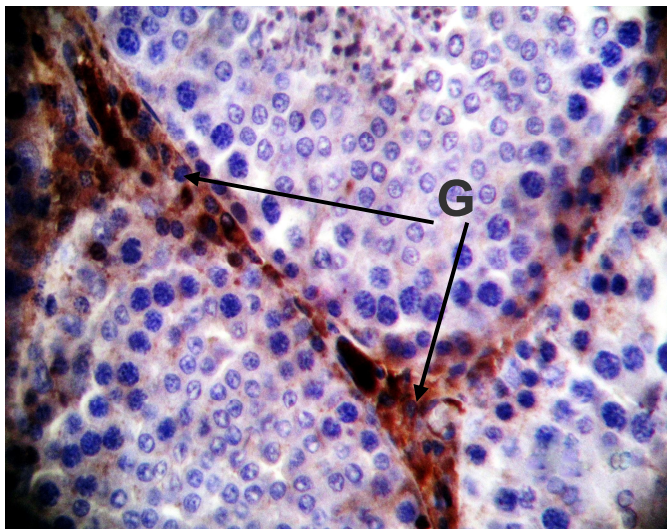


Figure (3): Section from diabetic rat testes after five months of diabetic duration showing marked increase in the GLUT3 protein (G),. (IHC ,X1000)

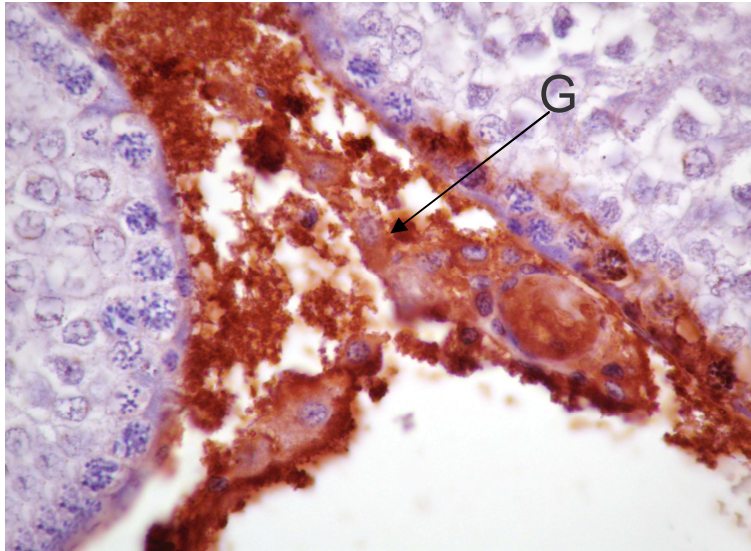


Figure (4):Section from normal rat testes after five months of experimental duration showing marked increase in the GLUT8 protein (G) (.IHCx1000)

Immunohistochemical localization of GLUT3 , GLUT8 proteins after treatment.

a. GLUT3 protein: the hypoglycemic effect of aqueous extract of *Cymbopogon citrates* on the activity of GLUT3 protein in normal and diabetes are shown in (table1) and (figures:5, 6, 7) in which the administration of this extract orally suppressed the elevation of GLUT3 protein significantly ($p \leq 0.05$) in all age groups with mean values (2.47) and (5.28) , while no significant effects were observed in non-diabetic treated groups as compared with normal control animals with mean values (2.38) and (5.12).

b. GLUT8 protein: the administration of *Cymbopogon citratus* to diabetic rats lead to an increase in the activity of glucose transporter 8 protein at ($p \leq 0.05$) in all age groups (7.6) and (14.7), while no significant effect was observed in normal animals treated with the herb with mean (7.68) and (14.72) compared with normal controls which received no treatment (figures 4).

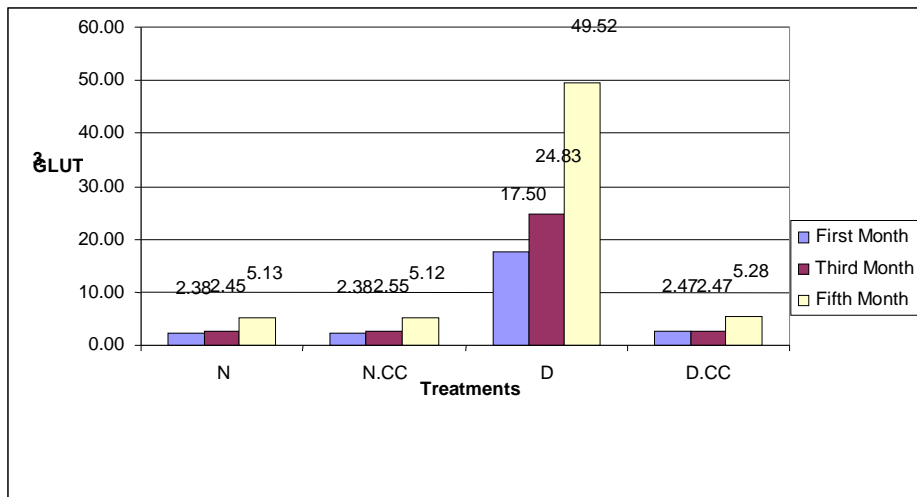


Figure (5): Effect of *Cymbopogon citrates* on the GLUT3 Protein in the testes of normal and alloxan induced diabetic group

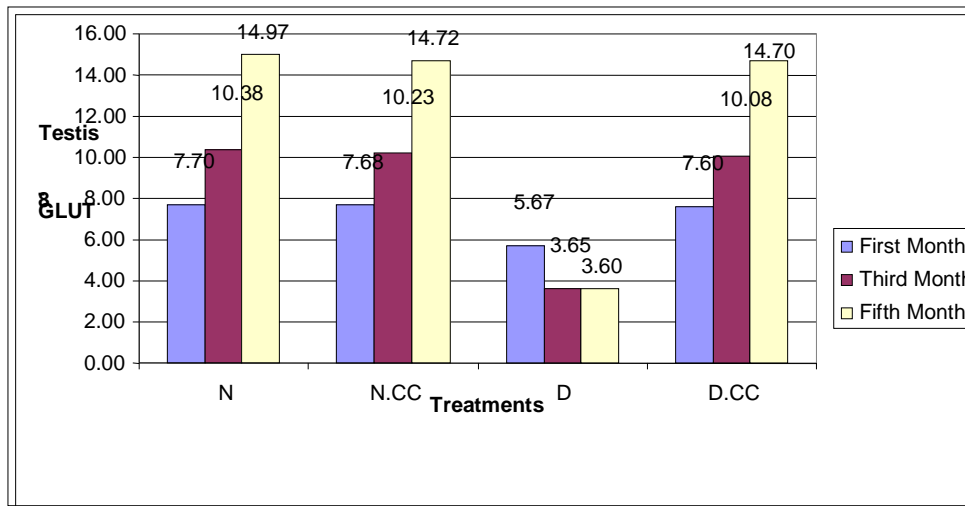


Figure (6): Effect of *Cymbopogon citratus* on the GLUT8 Protein in the testes of normal and alloxan induced diabetic group

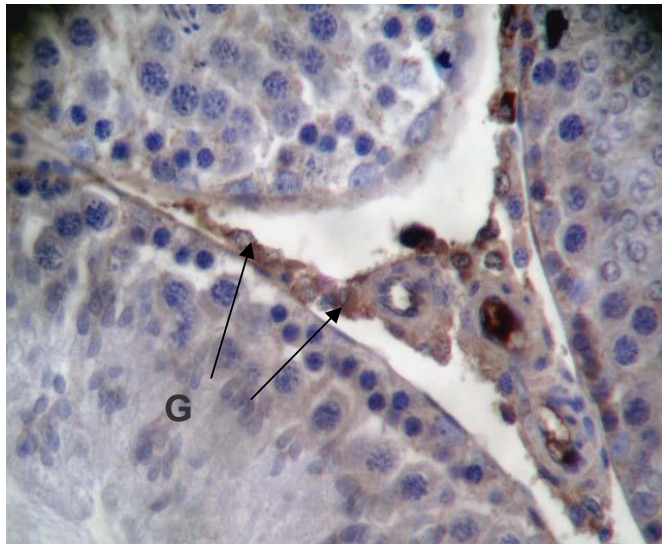


Figure (7):Section from diabetic rat testes after five months treatment with aqueous extract of *Cymbopogon citratus* showing marked decrease in the GLUT3 protein(G) (IHC ,X1000)

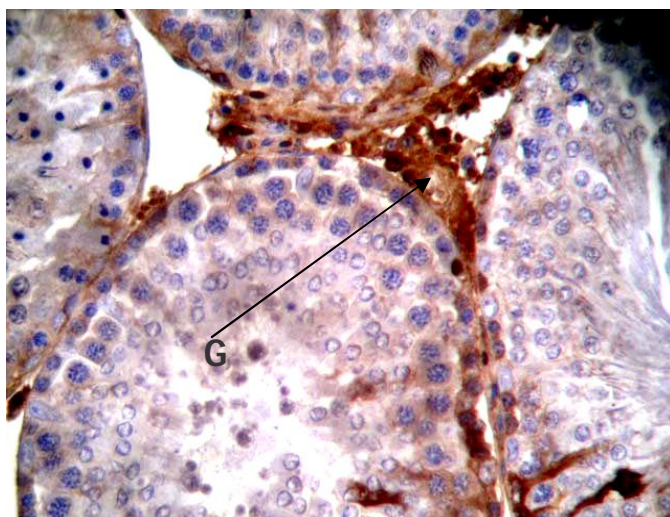


Figure (8):Section from diabetic rat testes after five months treatment with aqueous extract of *Cymbopogon citratus* showing marked increase in the GLUT8 protein with progressive time (G) (IHC ,X1000)

Table (1): the effect of aqueous extract of *Cymbopogon citrates* on the activity of GLUT3& GLUT8 protein in normal and diabetes

*	GLUT3			GLUT8-testes		
	First Month	Third Month	Fifth Month	First Month	Third Month	Fifth Month
N	2.38	2.45	5.13	7.70	10.38	14.97

	±	±	±	±	±	±
	0.08	0.10	0.05	0.37	0.86	0.05
	a	A	b	a	b	c
	17.50	24.83	49.52	5.67	3.65	3.60
D	±	±	±	±	±	±
	1.05	1.47	0.85	0.29	0.38	0.32
	a	B	c	b	a	a

If ($P \leq 0.05$) it mean significant difference

* *N = Normal* *D = Diabetes*

Discussion

The results indicate a significant increase in GLUT3 protein in testes in all diabetic groups with marked decrease in GLUT8 protein in testes in all age groups, these results are in agreement with previous studies obtained by Doege et al. (2000); and Kokk et al. (2004)[26,27]. An identical results obtained by Gorovits et al (2003) in that they found that during diabetes GLUT8 expression decreased and may linked to circulating LH and gonadotropins. It is described by Chen et al (2003) [28] that LH hormone stimulates GLUT8 mRNA in leydig cells, that is why during diabetes the decreases of LH causing a decreasing in GLUT8 protein expression. Such idea also supported by Ward et al. (1991)[29]; Shittu et al. (2006) [30], in that they said that diabetes lead to a marked decrease in the luteinizing hormone (LH) that controls leydig cells function which consequently leads to decreasing in GLUT8 protein expression.

The results showed that after administration of aqueous extract of Cymbopogon citratus enhances the activity of GLUT8 protein in the testes this may ascribed to; the fact that the main chemical components or active ingredients of lemongrass are: (myrcene, citronellal, geranyl acetate, nerol, geraniol, neral, traces of limonene and citral and beta-carotene) the later is a powerful anti oxidant: by which carotene reducing diabetic complications, like the glycosylation is the enzymatic process that links saccharides to produce glycans, either free or attached to proteins and lipids in alloxan induced diabetic rats[31], the other important components of this extract is citral which is necessary for vitamin A synthesis, in addition contain vitamin C which have

oxidant capacity [32,33]. The extract of the may has anti oxidant stress action and increase the tissue sensitivity and peripheral uptake of glucose lead to an increase in the hormone LH, when this hormone increases its effects on leydig cells lead to an increase GLUT8 protein.

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