A study on the effect of Chromium Administration on the Altered Urinary Bladder Reactivity in Experimentally-induced Diabetic Rats

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ABSTRACT

Diabetic-induced urinary bladder dysfunction (diabetic cystopathy) is among the most common complications of chronic uncontrolled diabetes mellitus. This cystopathy, involves altered urinary bladder contractility and reactivity, is suggested to be due to the oxidative stress encountered in diabetes. Chromium, an antioxidant micronutrient, is proposed nowadays as an adjuvant in some diabetic complications. Accordingly, this study was designed to evaluate the possible role of oral chromium administration on the altered urinary bladder reactivity in experimentally-induced diabetic rats. In the present study, 36 adult male albino rats, weighing 150-180 gm each, were used. They were divided into 6 equal groups. Group1 (Control group), Group2 (Cr-treated control group), Group3 (untreated diabetic group), Group4 (Insulin-treated diabetic group), Group5 (Cr-treated diabetic group) and Group6 (Concomitantly insulin & Cr-treated diabetic group). Fasting serum glucose, serum insulin, homeostatic model assessment to detect insulin resistance (HOMA-IR index), lipid profile, urinary bladder tissue malondialdehyde (MDA) (a tissue marker of oxidative stress and lipid peroxidation) and glutathione (GSH) (as an index of the tissue antioxidant enzyme defense activity) were measured. In addition, the total body weight, urinary bladder weight and the reactivity of isolated urinary bladder strips to carbachol (Cch) were determined. The untreated diabetic group exhibited significant increase of the serum glucose, HOMA-IR index, lipid profile, bladder tissue MDA and urinary bladder weight, (p<0.05) together with a significant decrease (p<0.05)of serum insulin, total body weight and bladder tissue GSH level. In addition, the contractile response of the isolated urinary bladder strips to Cch was significantly higher in untreated diabetic rats relative to the other tested groups, (p<0.05). Interestingly, when the diabetic rats were treated with subtherapeutic doses of insulin alone or oral chromium alone, a significant improvement (p<0.05) of all the altered parameters was obtained. However, when the diabetic rats were administered concomitantly with insulin & chromium, a highly significant improvement (p<0.001) of the deteriorated parameters that have been returned nearly close to the control level. In conclusion, oral chromium administration has a beneficial effect in ameliorating the changes in the urinary bladder reactivity in experimentally-induced diabetic rats, probably through its antioxidant effect and its ability in increasing insulin sensitivity.

INTRODUCTION

Diabetes mellitus (DM) is a metabolic disorder with multiple serious complications that involve almost all organs of the body. Diabetic cystopathy is one of the most common complications of chronic

DM[1]. The classic symptoms of diabetic cystopathy are decreased bladder sensation, increased bladder capacity and impaired bladder emptying with resultant increased post-void residual urine[2]. Also, this cystopathy usually involves altered urinary bladder contractility and reactivity[3]. It has been reported that it is difficult to restore the bladder function in diabetic patients[4]. Therefore, the development of new therapeutic approaches for such complication is ultimately needed.

Oxidative stress and production of reactive oxygen species (ROS) are implicated in the pathophysiology of most of diabetic complications in particular the diabetic cystopathy[5-6]. The involvement of ROS in the pathogenesis of diabetic cystopathy paid the attention for some antioxidants in the management of this deleterious consequence.

Chromium is one of these antioxidants that is proposed as an adjuvant in the management of diabetes and its complications[7]. Chromium is a micronutrient trace element found in mushroom, yeast, processed meats, broccoli and date. It has many valences ranged from Cr^{+2} to Cr^{+6}. It is interestingly noted that chromium in the trivalent form (Cr^{+3}) is an essential trace element for human and animals. However, its hexavalent form (Cr^{+6}) is found to be toxic, causing dermatitis, liver damage and renal necrosis[7].

The role of chromium in diabetes has been advocated from the observation that patients on long-term total parenteral nutrition (TPN), a solution known by its chromium deficiency, developed impaired glucose tolerance. Thus, nowadays, chromium is routinely added to all TPN solutions[8]. Moreover, it was found that chromium deficiency in rats and in humans led to a diabetic-like state of hyperglycemia, impaired glucose tolerance and relative insulin resistance[9]. These observations paid the attention of a possible link between chromium and the pathogenesis of diabetes. Also, they raised a question whether using chromium is of value in diabetic patient or not.

In that issue little trials have been established in the literature in which chromium was used as adjuvant in some diabetic cardiovascular complications e.g hypertension and changes of vascular reactivity[10] and in diabetic nephropathy[11].

Despite of these studies, yet, it is worthily noted that no attempts have been established in the literature considering the effect of chromium on diabetic cystopathy. Therefore, the present study was conducted to elucidate whether utilizing oral chromium will ameliorate such complication or not.

MATERIAL & METHODS

Animals

In the present study, 36 adult male albino rats of local strain, weighing 150-180 gram each, were used. Rats were housed in a standard condition of housing, controlled environment, kept on standard laboratory chow diet and had free access to water throughout the whole study period.

Experimental protocol

The rats were divided into 6 equal groups.

1- Control group:

In this group, each rat was subjected to a single intraperitoneal (I.P) injection of 0.5 ml of the vehicle, sodium citrate buffer solution, (50mM, pH 4.5). Then they were administered orally with 0.5 ml distilled water daily for 6 weeks.

2- Cr-treated control group:

In this group, each rat was subjected to a single I.P injection of 0.5 ml sodium citrate buffer solution (50mM, pH 4.5). Then they were administered orally with aqueous trivalent chromium picolinate solution (80 μgm/kg body weight (BW) dissolved in 0.5 ml distilled water, daily for 6 weeks)[9]. Chromium was obtained as capsules containing 200 μgm pure crystalline trivalent chromium picolinate/capsule (Amoun Pharmaceutical Co., Egypt) that were reconstructed by dissolving them in distilled water.

3- Untreated diabetic group:

Experimental diabetes mellitus was induced in this group by a single I.P injection of freshly prepared streptozotocin (STZ) solution (Sigma chemical Co., USA) (45mg/kg BW dissolved in 0.5 ml sodium citrate buffer solution (50mM, pH 4.5)[12]. Thereafter, the rats were administered orally with 0.5 ml distilled water daily for 6 weeks.

The fasting serum glucose level of the rats of this group was measured twice weekly, via a rat-tail sampling, throughout the whole study period and the rats were considered diabetic when the fasting serum glucose level was more than 120 mg/dl, 48 hours after induction of diabetes[13].

4- Insulin-treated diabetic group:

In this group rats were rendered diabetic as in the 3rd group and then they were immediately injected subcutaneously (S.C.) with a subtherapeutic dose of protamine zinc insulin (Eli Lilly Company (Indianapolis, IN) (1 IU/rat, daily for 6 weeks)[14].

5- Cr- treated diabetic group:

In this group, diabetic rats were administered orally with the trivalent chromium picolinate solution (80 μgm/kg BW daily, for 6 weeks).

6- Concomitantly insulin & Cr-treated diabetic group:

In this group, diabetic rats were concomitantly treated with both protamine zinc insulin (1 IU/rat daily) and chromium picolinate solution (80 μgm/kg BW daily) for 6 weeks.

Experimental procedures

1- Preparation of isolated urinary bladder strips[3]:

At the end of the experimental protocol (after 6 weeks), the rats of each group were subjected to overnight fasting, after which they were weighted and anesthetized by I.P sodium pentobarbital (40mg/kg BW), then blood samples were withdrawn from the retro-orbital veins at the medial epicanthus. Thereafter, a midline abdominal incision (labarotomy) was done and the abdomen was opened and the urinary bladder was rapidly dissected free from adherent fats, connective tissues and peritoneum and rapidly excised out of the body to be weighted and cut longitudinally into 2 strips at the level of urethral opening. One of these two strips was used for recording the

spontaneous urinary bladder contractions and reactivity while, the second one was stored and freezed at -80°C and was used later for determination of MDA and GSH levels in the bladder tissue.

2- Recording the basal spontaneous urinary bladder contractions[15]:

The first excised strip was immersed immediately into a warm oxygenated Krebs-Henseleit physiological solution (of composition in mmol/L: NaCl, 118.0; KCl, 4.7; KH2 PO4, 1.2; NaHCO3, 25.0; MgSO4, 1.2; CaCl2, 2.52; and glucose, 10.0 -pH adjusted to 7.4) that maintained at 37 ºC, and continuously aerated with carbogen (95% O2 and 5% CO2) to maintain pH 7.4.Then, this strip was tied at both ends with fine silk sutures and mounted along its longitudinal axis in a 30 ml organ bath containing the Krebs' solution.

The lower end of this isolated strip was connected to a hook fixed in an aeration tube while the upper end was connected to another hook suspended from a side way ink-writing lever that traced on a slowly rotating fixed-speed drum (5mm/min.) of a universal kymograph (Bioscience, USA). This suspended preparation was left to equilibrate in the organ bath for 60 min. during which the Krebs' solution was periodically washed every 15 minute to be replaced by a freshly prepared one. At the end of the equilibration period, the preparation was subjected to a resting tension of 1 gm via a load of one gram suspended at the lever side and the amplitude of the basal spontaneous rhythmic contractions (basal tone) of this strip was traced (in mm/gm wet bladder tissue) before it was challenged with graded concentrations of carbachol.

3- Testing the reactivity of the isolated urinary bladder strips to carbachol[16].

The reactivity of the previous mounted strip to carbachol (a parasymathomimetic drug, BDH Chemicals Ltd, England) was tested by adding this active agent in an increasing dose manner (10^{-8}, 10^{-7}, 10^{-6}, 10^{-5} & 10^{-4} M/ml bathing fluid). Upon adding each dose, a contractile response was traced for 5 min. after which the preparation was washed and left to return to the basal tone. and to equilibrate for 30. Min. Then a basal line was drawn for 5 min then another dose was added. From the obtained tracing, a log dose response curve was constructed. From this curve, EC50 (effective concentration that produces half maximal response) was calculated for each group separately. The EC50 was used in comparing the reactivity of the urinary bladder among different groups[17].

4- Determination of the bladder tissue MDA and GSH levels

On the day of the measurement, the second-freezed bladder strip was blotted dry and minced then homogenized in a glass homogenizer containing 10 ml of ice-cold phosphate buffer saline (PBS) of the following composition, KCl 140 mmol/L and phosphate 20 mmol/L, pH 7.4. Thereafter, the homogenized bladder tissue was centrifuged at 3000 round per minute (rpm) for 30 min. and the separated supernatant was used to estimate the bladder tissue MDA (μmol/gm wet tissue) and GSH (μmol/gm protein) by the methods

adopted by Oner-Iyidgan et al.[18] and Senft et al.[19] respectively.

Biochemical analysis

Fasting serum glucose[20], fasting serum insulin[21], fasting serum triglycerides (TG) and total cholesterol (TC)[20] and high density lipoprotein (HDL)[22] were measured. Also, serum low density lipoprotein (LDL) was calculated according to the formula of {LDL= TC- (HDL+TG/5) adopted by Friedwald et al.[23]. Insulin resistance was calculated by Homeostatic model Assessment-Insulin resistance (HOMA-IR index) according to the equation of

$$\text{HOMA-IR} = \frac{\text{Glucose} \times \text{Insulin}}{405} \text{ [24]}.$$

Where: glucose was in (mg/dL) and insulin was in (μU/ml).

Statistical analysis

All results were presented as mean ± standard error of means (SEM). One way analysis of variance (one-way ANOVA) followed by and further comparisons done by means of 'Student's t-test' for unpaired data.. The criterion for significance was set at P value <0.05[25].

RESULTS

In the present study, compared with the control group, the Cr-treated-control group exhibited a non-significant change (p>0.05) in the mean values of almost all the measured parameters except the mean total body weight that increased by 34%, in the control group whereas that of the Cr-treated group increased by 60% (P< 0.05 vs control) (table1-5 and fig.1-5).

In addition, compared with the control group, the untreated diabetic group, showed a significant increase (p<0.05) of the mean values of serum glucose (mg/dl) (86.64±5.24 vs 320.43 ±15.81), TG (mg/dl) (79.43±3.10 vs 115.67±5.53), TC (mg/dl) (72.32±4.83 vs 109.57±6.67), LDL(mg/dl) (35.25±3.46 vs 66.53±5.58), HOMA-IR index (4.66±0.2 vs 7.24±0.3), urinary bladder MDA (μmol/gm tissue) (5.32±0.03 vs 22.44±0.19), urinary bladder weight (mg) (80.61±4.11 vs 160.33±8.01), and the reactivity of isolated urinary bladder strips to carbachol represented by EC50 (2×10^{-8} vs 3×10^{-6}) (table1,2,3&5 and fig 1, 3&7).

On the other hand, comparing the results of the control group with those of untreated diabetic group, there was a significant decrease (p<0.05) of the mean values of the serum insulin (μU/ml) (24.23±2.6 vs 10.17±0.55), HDL (mg/dl) (35.25±3.46 vs 5.85±0.07), mean % change of the total body weight (increase 34% vs decrease 38%) and urinary bladder GSH (μmol/gm protein) (0.43 ±0.03 vs 0.12 ±0.01) (table 1-4).

Interestingly, in the present work, compared with the untreated diabetic group, the insulin-treated diabetic, and Cr-treated diabetic control groups demonstrated a significant increase (p<0.05), however, the concomitant insulin & Cr-treated diabetic group showed a highly significant increase (p<0.001) of the mean values of the serum insulin (μU/ml) (10.17 ±0.55 vs15.72±0.9, 16.11±1.08 and 21.65 ±1.23), HDL (mg/dl) (5.85±0.07 vs 10.11±0.06, 11.06±0.01 and 13.11±0.02), mean % change of the total body weight (decrease 38% vs decrease 19%, decrease 0.5% and decrease 0.4%) and urinary bladder

GSH (μmol/gm protein) (0.12±0.01, 0.20±0.01, 0.19±0.01, 0.37±0.02) (table1-4).

Also, when compared with the untreated diabetic group, the insulin-treated diabetic, and Cr-treated diabetic control groups demonstrated a significant decrease (p<0.05), however, the concomitant insulin & Cr-treated diabetic group showed a highly significant decrease (p<0.001) of the mean values of the serum glucose (mg/dl) (320.43±15.8 vs 130.28±5.88,   150.92±7.52   and 99.18±5.37),   TG   (mg/dl) (115.67±5.53   vs   92.11±4.58, 95.45±6.22   and   83.85±4.89),   TC (mg/dl) (109.57±6.67 vs 91.67±5.30, 99.40±6.18 and 80.11±3.03), LDL (mg/dl) (66.53±5.58 vs 46.34±3.88, 50.27±2.11and 38.55±2.94), HOMA-IR index (7.24±0.3 vs 4.55±0.1, 4.39±0.2 and 4.22±0.1), urinary bladder MDA (μmol/gm tissue) (22.44±0.19   vs   14.44±0.08, 15.32±0.02 and 5.37±0.15), urinary bladder weight (mg) (160.33±8.01 vs 110.21±6.45,   157.55±7.90   and 88.05±5.22) and the reactivity of isolated urinary bladder strips to carbachol represented by EC50(2×10^{-8} vs 2×10^{-7}, 2×10^{-7} and 4×10^{-6}) (table1-5 and fig 3-7).

Table (1): Fasting serum glucose, serum insulin and HOMA-IR in all the studied groups of rats

	Control group	Cr-treated control group	Untreated diabetic group	Insulin-treated diabetic group	Cr-treated diabetic group	Concomitantly insulin & Cr-treated diabetic group
Serum glucose (mg/dl)	86.64 ±5.24	80.22 ±4.55	320.43* ±15.81	130.28*● ±5.88	150.92*● ±7.52	99.18● ±5.37
Serum insulin (μU/ml)	24.23 ±2.6	22.41 ±3.55	10.17* ±0.55	15.72*● ±0.91	16.11*● ±1.08	21.65● ±1.23
HOMA-IR	4.66 ±0.2	4.34 ±0.1	7.24* ±0.3	4.55● ±0.1	4.39● ±0.2	4.22● ±0.1

Values are represented as mean ±SEM   Statistical significant difference is set at P<0.05

* = Significant when compared with the control group

● = Significant when compared with the untreated diabetic group

Table (2): Serum TG, TC, HDL and LDL in all the studied groups of rats

	Control group	Cr- treated control group	Untreated diabetic group	Insulin- treated diabetic group	Cr- treated diabetic group	Concomitantly insulin & Cr-treated diabetic group
TG (mg/dl)	79.43 ±3.10	81.58 ±2.44	115.67* ±5.53	92.11*● ±4.58	95.45*● ±6.22	83.85● ±4.89
TC (mg/dl)	72.32 ±4.83	70.31 ±4.57	109.57* ±6.67	91.67*● ±5.30	99.40*● ±6.18	80.11● ±3.03
HDL (mg/dl)	14.44 ±1.06	13.72 ±1.04	5.85* ±0.07	10.11* ● ±0.06	11.06* ±0.01	13.11● ±0.02
LDL (mg/dl)	35.25 ±3.46	32.77 ±2.81	66.53* ±5.58	46.34*● ±3.88	50.27*● ±2.11	38.55● ±2.94

Values are represented as mean ±SEM   Statistical significant difference is set at $P<0.05$

* = Significant when compared with the control group

● = Significant when compared with the untreated diabetic group

Table (3): Urinary bladder weight, bladder tissue MDA and bladder tissue GSH in all the studied groups of rats

	Control group	Cr- treated control group	Untreated diabetic group	Insulin- treated diabetic group	Cr- treated diabetic group	Concomitantly insulin & Cr-treated diabetic group
Urinary bladder weight (mg)	80.61 ±4.11	82.84 ±3.64	160.33 ±8.01	110.21*● ±6.45	157.55*● ±7.90	88.05*● ±5.22
Bladder tissue MDA (μmol/gm tissue)	5.32 ±0.03	4.57 ±0.01	22.44* ±0.19	14.44*● ±0.08	15.32*● ±0.02	5.37● ±0.15
Bladder tissue GSH (μmol/gm protein)	0.43 ±0.03	0.49 ±0.03	0.12 ±0.01	0.21*● ±0.01	0.19*● ±0.01	0.37*● ±0.02

Values are represented as mean ±SEM   Statistical significant difference is set at $P<0.05$

* = Significant when compared with the control group

● = Significant when compared with the untreated diabetic group

Table (4): The mean % change in the total body weight of rats at the end of the experimental period (6 weeks) in all the studied groups of rats

	Control group	Cr-treated control group	Untreated diabetic group	Insulin-treated diabetic group	Cr- treated diabetic group	Concomitantly insulin & Cr-treated diabetic group
Initial body weight (gm)	160.32 ±6.73	162.58 ±6.44	163.72 ±5.98	161.67 ±5.99	160.12 ±8.08	162.32 ±7.65
Final body weight (gm)	215.42 ±9.12	260.77 ±12.54	100.91 ±11.82	130.32 ±9.93	151.64 ±7.92	155.37 ±5.61
% change in body weight	+ 34%	+ 60%*	- 38%*	- 19%*●	- 0.5%*●	- 0.4.%*●

Values are represented as mean ±SEM   Statistical significant difference is set at P<0.05

* = Significant when compared with the control group

● = Significant when compared with the untreated diabetic group

(+) means increase   (-) means decrease

Table (5): The mean EC50 values of different doses of Cch (10^{-8} - 10^{-4} Mol) on the isolated urinary bladders in all the studied groups of rats

	Control group	Cr-treated control group	Untreated diabetic group	Insulin-treated diabetic group	Cr- treated diabetic group	Concomitantly insulin & Cr-treated diabetic group
EC50	3×10^{-6}	4×10^{-6}	2×10^{-8}*	2×10^{-7}*●	2×10^{-7}*	4×10^{-6}●

* = Significant when compared with the control group

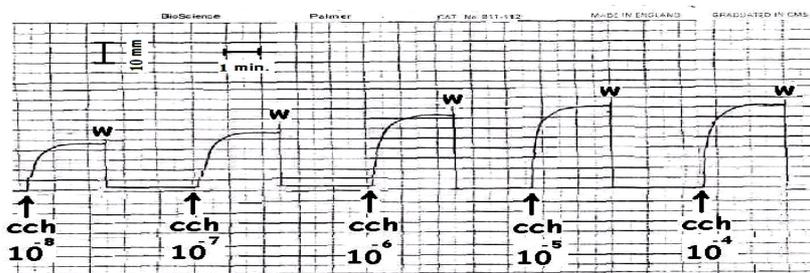● = Significant when compared with the untreated diabetic group

Fig. (1): Tracing showing the amplitude of the urinary bladder contraction (mm/gm wet tissue) to different doses of carbachol (Cch) in the control group of rats
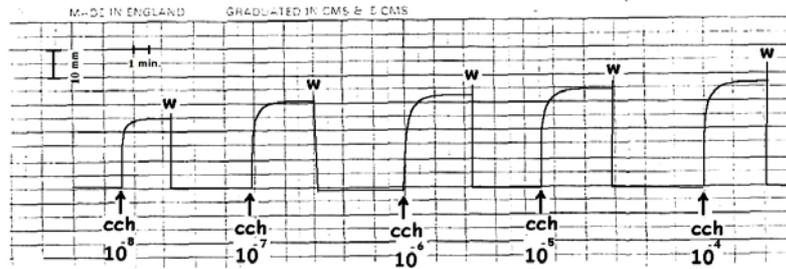
Fig.(2): Tracing showing the amplitude of the urinary bladder contraction (mm/gm wet tissue) to different doses of carbachol (Cch) in the Cr-treated control group of rats
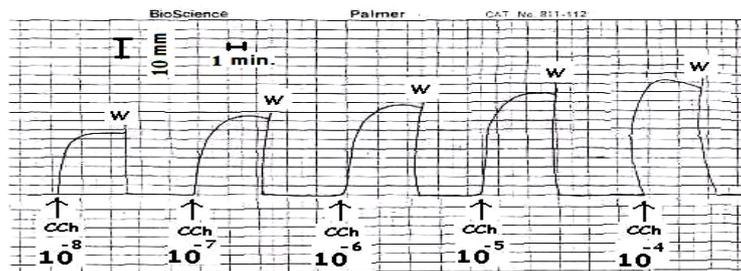

Fig.(3): Tracing showing the amplitude of the urinary bladder contraction (mm/gm wet tissue) to different doses of carbachol (Cch) in the untreated diabetic group of rats
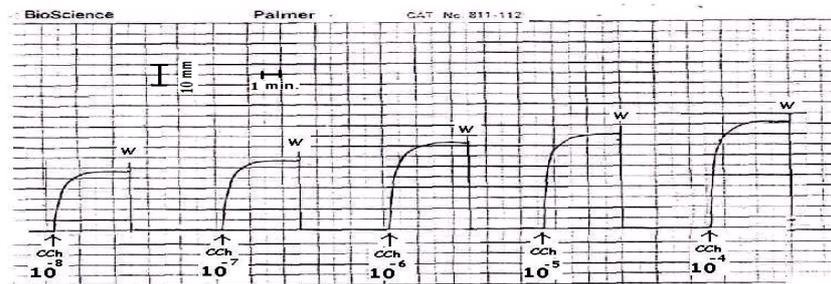

Fig.(4): Tracing showing the amplitude of the urinary bladder contraction (mm/gm wet tissue) to different doses of carbachol (Cch) in the insulin-treated diabetic group of rats
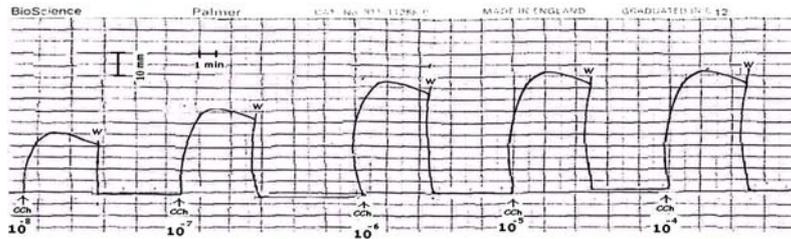
Fig. (5): Tracing showing the amplitude of the urinary bladder contraction (mm/gm wet tissue) to different doses of carbachol (Cch) in the Cr-treated diabetic group of rats
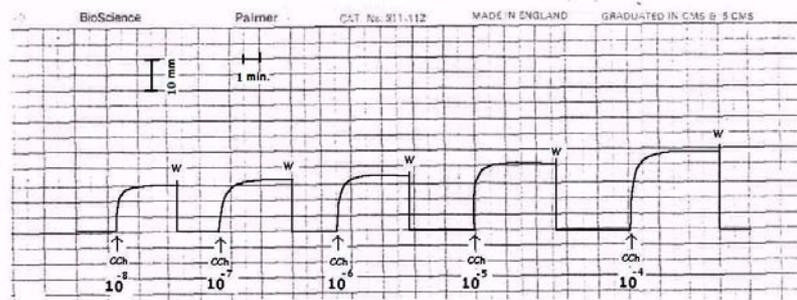

Fig.(6): Tracing showing the amplitude of the urinary bladder contraction (mm/gm wet tissue) to different doses of carbachol (Cch) in the concomitant insulin & Cr-treated diabetic group of rats
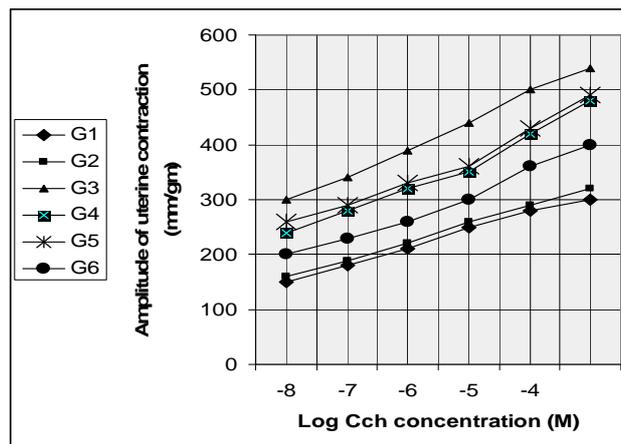

Fig. (7): Log dose-response curve of Cch (10^{-8}- 10^{-4}) on the amplitude of urinary bladder contraction (mm/gm wet tissue) taken from different tested groups
G1: Control group   G2: Cr-treated control group   G3: untreated diabetic group
G4: Insulin-treated diabetic group   G5:Cr-treated diabetic group
G6: Concomitantly insulin & Cr-treated diabetic group

DISCUSSION

In the present study, the untreated diabetic rats exhibited a statistical significant increase of the mean value of fasting serum glucose and a significant decrease in the mean value of serum insulin when compared with that of the other groups.

These results agreed with Jiang et al.[26] who found increase serum glucose in STZ-induced diabetic rats. Interestingly, in the present work, when the diabetic rats were supplemented orally with chromium alone or subtherapeutic doses of insulin alone, a significant decrease in fasting serum glucose and a significant increase in serum insulin levels were obtained. However, when chromium and insulin were concomitantly administered, a highly significant decrease in fasting serum glucose and a highly significant increase in serum insulin were displayed.

The above-mentioned findings were agreed with Cefalu et al.[27] who found that oral chromium administration decrease blood glucose level and increase insulin level in STZ-induced diabetic rats. In addition, by an unknown mechanism, it was reported that chromium has no effect on normal serum insulin, but it has an insulin-rising effect in hypoinsulinemic rats[28].

Furthermore, Anderson et al.[29] found a significant decrease in serum glucose and improvement in glucose tolerance in diabetic subjects. Moreover, these finding were in accordance with Kim et al.[30] who found that oral chromium improved

partially the functions of beta cells of pancreas in diabetic rats consequently, the serum insulin level is mildly increased. They have attributed these effects of chromium to its insulin-potentiating effect.

The mechanism by which chromium potentiate the insulin action, is believed to be due to its ability in increasing the insulin sensitivity of the smooth muscles by a direct activation of tyrosine kinase activity of insulin receptors[27]. Another postulated mechanism is indirect activation of tyrosine kinase activity of insulin receptors by the chromodulin (Cr-binding substance) which is a low-molecular-weight oligopeptide widely distributed in almost all tissues and has four binding sites ready to bind four Cr ions in its metal-free form[8]. It was found that after chromium administration and absorption, it is taken up by insulin sensitive cells and combines with the intracellular chromodulin. This resulted in a conformational change of chromodulin into its active chromium-chromodulin complex. This active chromodulin binds to insulin-receptors and consequently activates their tyrosine kinase activity as much as eight folds[8]. In addition, it was reported that active chromodulin increased the glucose transporters specially Glut-4 on the muscle cells with subsequent enhancement of glucose transport into these cells and its subsequent utilization Also, the active chromodulin was reported to increase gene expression of insulin in the beta cells[9].

This role of chromium in "auto-amplification mechanism in insulin

signaling" might provide a new insight of how insulin action can be enhanced by chromium supplementation and provide a new trend in management of diabetes where chromium could be used in potentiating the action of insulin and decrease insulin resistance but it cannot act as a substitute of it[9].

Interestingly, insulin resistance, the impaired ability of tissues to respond efficiently to insulin, is the common denominator in many of metabolic conditions (hyperglycemia, hypertension, and hypertriglyceridemia, with concomitant reduced HDL and TC described as "syndrome X."[8]. In the present study, the decrease of insulin resistance, as indicated by significant improvement of (HOMA-IR) index in the diabetic rats upon chromium supplementation, could be explained by the results of Cefalu et al[27] who sited that chromium supplementation enhances insulin sensitivity and glucose disappearance rate.

In the present work, the diabetic rats showed a statistical significant increase in fasting serum levels of TG, TC and LDL with a significant decrease in fasting serum HDL when compared with the corresponding values in the other groups of rats. This disturbed lipid profile encountered in diabetes is mostly due to decreased serum insulin level and decrease in glucose uptake by peripheral tissues that ultimately led to increased lipolysis. Insulin is clearly a lipogenic hormone-facilitating fat synthesis and storage. Thus, chromium's insulin-enhancing action may be expected to promote weight gain and increase fat mass rather than fat reduction[31].

The elevated level of LDL in diabetic rats may be due to a decrease in the uptake of LDL by its receptor molecules. This may be due to conformational change in the structure of these molecules caused by their glycation that occur due to hyperglycemia. On the other hand, the decreased HDL in diabetic rats may be explained on the basis of the known inverse correlation of HDL with TG level and a positive correlation with lipoprotein lipase activity[31].

The elevated LDL together with oxidative stress in diabetes mostly yield to the oxidized-LDL that is considered a potential risk factor for developing diabetic cystopathy and change in the urinary bladder reactivity[32].

On the contrary, when diabetic rats were supplemented by chromium alone or insulin alone a significant decrease in fasting serum TG, TC and LDL with a significant increase in serum HDL were noticed. However, when chromium & insulin were concomitantly administered, a highly significant attenuation of these lipid profile was displayed.

These findings were in consistent with Abraham et al.[33] and Amato et al.[34] who reported that chromium administration ameliorated diabetes-associated dyslipidemia in patients with type 2 and type 1 diabetes respectively. In addition, chromium, through its insulin-sensitizing action, was reported to decrease the elevated LDL and TC in diabetic rats[27]. The postulated mechanism of chromium in ameliorating the diabetic-induced dyslipidemia is through its ability in forming a compound called "niacin-

bound chromium". This compound was found to, by unsettled mechanism, decrease TC, LDL, oxidized LDL, body weight & ROS in humans[35].

The revealed results of this study showed that the diabetic rats displayed a significant decrease of the body weight. This agreed with Kocak et al.[10] and Jiang et al.[26] who reported that the body weight decreased significantly in diabetic rats. This decline in body weight could be explained by the loss of the anabolic effect of insulin hormone with subsequent increase of lipolysis[29]. Also, it may be due to decreased plasma proteins resulted from diabetic nephropathy or liver impairment that is usually encountered in cases of diabetes[33]. Moreover, the decreased body weight could be explained by the imbalance between antioxidant and oxidant state with dominance of the later in cases of diabetes.

On the other hand, when diabetic rats were supplemented by chromium alone or insulin alone a partial increase in the body weight was noticed. However, when chromium & insulin were concomitantly administered the body weight was returned nearly to the normal control value. These findings were consistent with kim et al.[36] who found that the decline in the body weight was improved in diabetic rats that were supplemented with chromium and they attributed these findings to the insulin-sensitizing action of chromium and its ability to potentiate insulin function in transporting amino acids inside the muscle cells. Also, Campbell et al.[37] found that concomitant insulin and chromium

administration increased the total plasma proteins, lean body mass and decreased the body fat in diabetic individuals.

Regarding the weight of the urinary bladder, the present study demonstrated that, the bladder weight increased significantly in diabetic rats compared with that of the other groups. These results were in support with Kodama and Takimoto[38] who reported a significantly higher urinary bladder weight than those of age-matched controls. Also, the increased diabetic bladder mass observed in the present study was in consonance with the findings of Tammela et al.[39] who found a significantly increased diabetic bladder mass in their study, and concluded that effects of diabetes on contractile bladder function are related to diuresis-induced increases in bladder mass.

The mechanism beyond this increased urinary bladder weight is the diabetic-induced detrusor muscle hypertrophy that represents a physiological adaptation to much increase in urine output in cases of diabetes In addition, diabetes is associated with a hypertrophy of the uretheral sphincters that increased the uretheral resistance to the urine flow that ultimately led to further hypertrophy of the detruser muscle[40]. Also, the urinary bladder is known to synthesize endothelin-1, which has a potent myogenic properties[41].

On the contradiction, oral supplementation of diabetic rats with chromium alone or insulin alone showed no significant change in bladder weight. However, concomitant chromium and insulin resulted in partial restoration of the

bladder weight back to the control level. This improvement in bladder weight could be explained by the partial restoration insulin and the balance between oxidant and antioxidant state inside the bladder tissue as was evidenced by a significant rise of bladder tissue MDA and decrease of GSH in this study.

Regarding oxidant/antioxidant status in the present study, the bladder tissue MDA increased while the bladder tissue GSH decreased significantly in the diabetic group compared with the other groups. Consistent with the these finding, Jiang et al.[26] have found a significant rise in plasma MDA and thiobarbituric acid reactive substances (TBARS, a marker of lipid peroxidation) and simultaneous decline of catalase capacity in plasma and intracellularly in the diabetic patients.

Additionally, Beshay and Carrier[42] have found a statistically significant increase of TBARS level in the bladders of the diabetic group compared with the control group. Also, Poladia and Bauer[43] have reported that the diabetes–induced bladder dysfunction in rats are mostly due to release of reactive nitrogen species that led to endothelial and smooth muscle changes of the urinary bladder. It has been sited that the hyperglycemia is contributed for the increased generation of free radicals in diabetes via an 'auto-oxidation glycosylation process' that leads to auto-oxidation of glucose.

Also, autoxidation process leads to autoxidation of free fatty acids, autoxidation of lipoproteins of the mitochondrial cell membrane and non-enzymatic glycation of proteins. These auto-oxidative reactions yield oxidizing intermediates and reducing molecular oxygen like superoxide anion (O^-_2), hydroxyl radical (OH^-) and hydrogen peroxide (H_2O_2)[8].

These diabetic–induced ROS are usually responsible for the damage of the intracellular organelles, denaturation of intracellular enzymes, increase lipid peroxidation of cell and nuclear membranes via formation of cytotoxic aldehyde that ultimately led to cell dysfunctions and may be cell apoptosis[44].

In the present work, oral supplementation of diabetic rats with chromium decreased the diabetes induced-lipid peroxidation of urinary bladder tissue as indicated by the reduction of MDA production and increased GSH in the bladder tissue. This beneficial role of chromium might be due to its sharing in the "niacin-bound chromium" that exhibit a preventive effect against oxidative stress[35].

Considering the basal spontaneous contractions of the isolated urinary bladder strips in the present study, the diabetic rats revealed a significant decrease in these contractions when compared with the other groups. In support with these obtained results, Gupta et al.[45] found a significant reduction in basal contractions of the isolated bladder strips of diabetic rabbits and they have resorted this to the decreased Na+-K+ ATPase activity in diabetes that led to increase intracellular Na+ concentration which in turn led to decrease Ca2+ influx via voltage-gated Ca2+ channels.

Also, the diabetic induced-vesical dysfunction, is suggested to be due to reduced availability of nerve growth factor (NGF)[46] or the autonomic diabetic neuropathy[2].

Beshay and Carrier[42] have found a significant decrease in the amplitude of the spontaneous contractions of the isolated urinary bladder strips of STZ-diabetic rats. They have attributed this to the increased level of TBARS and decreased the antioxidant scavenging enzymes catalase, superoxide dismutase and GSH in isolated urinary bladder strips. Moreover, Changolkar et al.[5] found a significant decrease in contractility of isolated detrusor smooth muscle in STZ-diabetic rats and they have resorted this bladder dysfunction to the concomitant increased of MDA level in bladder tissue.

The diabetic-induced decrease in the basal spontaneous contractions of the isolated urinary bladder is mostly related to increases generation of ROS. This is because these reactive species have the ability to oxidize polyunsaturated fatty acids (PUFA) in the cell membranes enhancing the lipid peroxidation of detrusor myocytes and mitochondria that resulted mostly to impaired detrusor muscle contractility and decrease in energy production[47].

Disagree with our results, Tarcan et al.[48] found that the basal urinary bladder contractions increased in the diabetic rabbits. They have attributed that to the rise of 8-iso-prostaglandin F2α (8-iso-PGF2α), in the urinary bladder (a marker of oxidative stress that causes potent urinary bladder contraction). This discrepancy in finding may be related to the difference in species of the experimental animals.

In the present investigation, urinary bladder strips of the diabetic rats developed more responsiveness to Cch than those obtained from the other groups. This agreed with previous work at which there was an increased responsiveness of isolated bladder strips of diabetic rats to bethanechol[49], carbachol[3] and acetylcholine[17].

The postulated mechanism of this enhanced reactivity of the bladder strips of diabetic rats is the increase of the total number of muscarinic-M3 receptors in the detruser muscle. This rise of muscarinic receptors is thought to be due to many factors. First, is the diabetic-induced bladder hypertrophy[49]. Second, it may be considered as a compensatory mechanism for diabetic neuropathy and the hypersensitivity of detrusor muscle to the muscarinic agonists[50]. Third, it may be due to up regulation of muscarinic receptors as an endothelial dysfunction that is thought to be due to rise of hydroxyl radicals and oxidized-LDL in diabetes[3].

Another postulated mechanism, for increased reactivity of the bladder strips of diabetic rats to Cch, is reported by Waring and Wendt[1] who contributed the high contractile responses of bladder strips to Cch in the diabetic rats to the increased influx of extracellular Ca^{2+} through the ligand-gated Ca^{2+} channels and they confirmed this by using the calcium antagonist "nifedipine" that caused a dose-dependent decrease in the contractile responses.

In the present work, on treating the diabetic rats with a sub-therapeutic

doses of insulin alone, the urinary bladder reactivity to Cch was attenuated partially. It is established that insulin partially attenuates the vascular reactivity to pressor agonists in vitro, accelerates vascular smooth muscle relaxation and enhances Ca^{2+}-ATPase-mediated cellular Ca^{2+} efflux[51].

Furthermore, on supplementing the diabetic rats with oral chromium alone, it attenuated partially the urinary bladder reactivity. This beneficial effect of oral chromium could be explained first, by its potentiating action on insulin[52]. Second, is via the improvement of the diabetic state as evidenced by decline of serum glucose, increase of serum insulin, and decrease insulin resistance and improvement of lipid profile in the diabetic rats.

Additionally, amelioration of the urinary bladder reactivity in the diabetic rats by chromium might be via its ability in restoring the bladder size back nearly to normal evidenced by the results of this work. Also, being a free radical-scavenging agent,[9] chromium could attenuate the bladder reactivity in the diabetic rats as noted by a significant decline of the bladder MDA content and augmentation of GSH production in this study.

Finally, concomitant insulin and chromium administration in the diabetic rats yield an additive and synergistic effects compared to insulin or Cr-treatment alone in enhancing the reactivity of urinary bladder to Cch.

In conclusion, oral chromium administration seemed to play an important role in attenuating the diabetic cystopathy and ameliorating the altered urinary bladder reactivity in experimentally-induced diabetic rats. This beneficial effect of chromium was probably ascribed to its insulin-potentiating effect and its antioxidant activity. These finding might provide a base for further clinical trials and expand the approach for the management of diabetes mellitus where chromium can be used in potentiating the action of insulin but it does not act as a substitute of it.

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دراسة عن تأثير عنصر الكروميوم علي بعض التغيرات في الاستجابة الانقباضية للمثانة البولية في الجرذان المحدث بهم مرض البول السكري تجريبيا

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من أكثر المضاعفات المعروفة لمرض البول السكري هو الاضطراب الوظيفي للمثانة البولية الذي يؤثر علي الاستجابة الانقباضية وتفاعلية المثانة لبعض محفزات مستقبلات المسكرين. ويعزي العلماء ذلك الخلل الوظيفي إلي زيادة نسبة الشوارد الحرة والأكسدة بالجسم.

ويهدف هذا البحث إلي استخدام عنصر الكروميوم (كاحدي مضادات الأكسدة) في الوقاية من حدوث الاضطراب الوظيفي للمثانة البولية في الجرذان المحدث بهم مرض البول السكري تجريبيا.

وقد اجري هذا البحث علي ٣٦ من ذكور الجرذان والتي تم تقسيمهم إلي ٦ مجموعات متساوية.

المجموعة الأولي: وهي مجموعة ضابطة.

المجموعة الثانية: وهي مجموعة ضابطة تم إرواءهم بعنصر الكروميوم عن طريق الفم (٨٠ ميكروجرام/كجم من وزن الجسم يوميا) لمدة ٦ أسابيع.

المجموعة الثالثة: وهي مجموعة الجرذان المحدث بها مرض البول السكري تجريبيا عن طريق حقنهم بالغشاء البيروتوني بجرعة واحدة من الاستربتوزوتوسين (٤٥ مجم/كجم من وزن الجسم مذاب في محلول سترات الصوديوم).

المجموعة الرابعة:وهي مجموعة من الجرذان المصابة بالبول السكري تم حقنهم بالانسولين (وحدة واحدة تحت الجلد يوميا) لمدة ٦ أسابيع.

المجموعة الخامسة: وهي مجموعة من الجرذان المصابة بالبول السكري تم إرواءهم بعنصر الكروميوم عن طريق الفم (٨٠ ميكروجرام/كجم من وزن الجسم يوميا) لمدة ٦ أسابيع.

المجموعة السادسة: وهي مجموعة من الجرذان المصابة بالبول السكري تم حقنهم بالانسولين (١ وحدة تحت الجلد) مع إرواءهم بعنصر الكروميوم عن طريق الفم (٨٠ ميكروجرام/كجم من وزن الجسم) يوميا لمدة ٦ أسابيع.

وقد أظهرت النتائج علي تأثيرات ضارة لمرض البول السكري تمثلت في زيادة ذات دلالة إحصائية في مستوي الجلوكوز والدهون الثلاثية والكوليستيرول والبروتينات الدهنية ذات الكثافة الخفيفة بالدم ومؤشر مقاومة الانسيولين ونسبة الدهون المؤكسدة (المالونداهيد) بنسيج المثانة البولية وكذلك وزنها. بالإضافة إلي زيادة تفاعلية شرائح المثانة البولية المعزولة للكارباكول (احدي محفزات مستقبلات المسكرين). وكذلك أدي مرض البول السكري الي نقص ذات دلالة إحصائية في نسبة الانسولين والبروتينات الدهنية ذات الكثافة العالية بالدم ووزن الجسم ونسبة الإنزيم المضاد للأكسدة (الجلوتاثيون) بنسيج المثانة البولية.

وقد أدي حقن الفئران المصابة بمرض البول السكري بالانسولين فقط أو إرواءهم بالكروميوم فقط إلي تحسن ذو دلالة إحصائية في معظم القياسات. بينما أدي البول السكري بالانسولين والكروميوم معا إلي تحسن ذو دلالة إحصائية كبيرة في معظم القياسات تقريبا إلي المستوي الموجود في جرذان المجموعة الضابطة.

ومن تلك الدراسة نستنج أن عنصر الكروميوم يساعد في الوقاية من حدوث الاضطراب الوظيفي للمثانة البولية في مرض البول السكري وذلك لأنه يساعد علي عمل هرمون الانسولين ويزيد من فاعليته والاهم من ذلك انه مضاد للأكسدة.