

Study the Effects of Glutathione (GSH) on Blood Glucose Level in Diabetic Male Mice

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Abstract

Investigated effects of daily injections intra venially (IV) of glutathione (GSH) (11.37 mg/mice) for the periods 10 and 20 days, for healthy mice and diabetic male mice compared them with control group and diabetic non treated male mice, blood sugar was tested for fasting mice, induced diabetic, to study the glutathione daily injections effects on the blood sugar level, total glutathione assay in different body organs and finally study the expression level of the glutathione redox system enzymes glutathione reductase (GR) and glutathione peroxidase (GPx) in pancreas, spleen, testis, and epididymus by the Reverse Transcriptase Polymerase Chain Reaction (RT-PCR). The experiment outcomes for the glutathione daily injection for the diabetic induced male mice high sugar level significantly decreased. Moreover, the oxidative stress in diabetic male mice leads to depletion of total glutathione level in body organs. Finally, Diabetes causes high oxidative stress in male mice which leads to increase expressions of antioxidants enzymes GR and GPx in pancreas, epididymus and testis.

Keywords: Glutathione, diabetic mice, blood glucose level.

Introduction

The existence of experimental animal model of a disease aids not only the understanding of the pathophysiology of such disease, but also the development of drugs for its treatment [1]. Due to its high prevalence and potential deleterious effect on a patient physical and psychological state, diabetes is a major medical concern [2]. The disease remains incurable and can only be controlled with drugs. Over the years, several animal models have been developed for studying diabetes mellitus or testing anti-diabetic agents. These models include chemical, surgical (pancreatectomy) and genetic manipulations in several animal species to induce diabetes mellitus. The diabetogenic drugs used such as alloxan monohydrate, which have been used in this experiment [1].

Enzymatic antioxidants are also known as natural antioxidants; they neutralize excess ROS and prevent it from damaging the cellular structure. Enzymatic antioxidants are composed of superoxide dismutase (SOD), catalase, glutathione peroxidase (GPx), and glutathione reductase (GR) which also causes reduction of hydrogen peroxides to water and

alcohol; diabetics have high levels of oxidative stress, which basically means too many free radicals and not enough antioxidants to neutralize them [3]. Diabetics also have low levels of intracellular glutathione (GSH) The high levels of oxidative stress and the low (GSH) levels further complicates the diabetic state which leads to even higher levels of oxidative stress and even lower levels of GSH [4]. Glutathione, being the master antioxidant, would naturally be the best choice as an antioxidant. Furthermore, inflammation leads to and contributes to insulin resistance. Glutathione, on top of being the most potent antioxidant, is also a powerful anti-inflammatory [5]. Oxidative stress plays a major role in the pathogenesis of both types of diabetes mellitus. Free radicals are formed disproportionately in diabetes, abnormally high levels of free radicals and the simultaneous decline of antioxidant defense mechanisms can lead to development of insulin resistance. These consequences of oxidative stress can promote the development of complications of diabetes mellitus [3], there is evidence for increased oxidative stress in diabetes, With regard to diabetes, antioxidants

supplementation have been shown to be beneficial. Thus, it appears that, in diabetes, antioxidant therapy could alleviate the increased attendant oxidative stress and emerge as an additional therapeutic modality, inflammation leads to insulin resistance and Type 2 diabetes [5], two ATP molecules are consumed in the synthesis of one GSH molecule, the spontaneous decline of GSH and the GSH-depleted cells generally appear to be healthy. This is because Cystein levels increase in response to the low GSH as a compensatory reaction and maintain a reduced cellular state [6].

Experimental Work

Experimental Animals

Male albino mice (*Mus musculus*) of age six months were kept in aluminum and plastic cages of dimensions 260 mm x 200 mm x 140 mm they were supplied with normal mice food and water *Ad libitum*. Mice were provided by ("Mario Negri" Institute for Pharmacological Research, Milano, Italy) and were bred at Charles River Italia (Calco, Lecco, Italy). Mice were housed at a temperature of $21 \pm 1^\circ\text{C}$ with relative humidity of $55 \pm 10\%$ and 12 hours light/dark cycle. Procedures involving animals and their care were conducted according to international laws and policies [7]. The animals were grouped into four groups, each group consisting of six mice subdivided to two periods of time 10 days and 20 days of experiment total period. Group 1: negative control (A) group, Group 2: diabetic group + GSH (B10 and B20) diabetic group treated IV daily with GSH 10 days and another subgroup 20 days, Group 3: diabetic group (C10 and C20) diabetic mice for two periods of disease, Group 4: positive group + GSH (D10 and D20), healthy mice injected daily for two periods of time 10 and 20 days. Mice were sacrificed by cervical dislocation.

Diabetes mice induction [8]

After 4 hours fasting normal non diabetic mice sugar blood level was 100 mg/dl (60-130) mg/dl. Inductions of diabetes in mice were as follow:

1. Fourty eight Hours fasting.
2. IP Alloxan injection in 150 mg /kg body weight.
3. After 30 min. of injection ended the fasting period.
4. After 10 days of the injection the blood sugar level were checked.

Mice blood sugar determination [8]

Blood samples were taken from the tail and blood glucose levels were determined (ACCU-CHEK; Roche Diagnostics, France). The bloods were taken as in the Table (1)

Table (1)
Blood sampling times of mice groups.

| group | Blood samples |
|-------|--|
| A | Mice blood samples before fasting / after fasting |
| B | Mice blood samples before fasting / after fasting / after 10 days of Alloxan injection / [B10(after 10 days of GSH treatment)/ B20 (after 20 days of GSH treatment)] |
| C | Mice blood samples before fasting / after fasting / after 10 days of Alloxan injection / [C10 (after 10 days of diabetes)/ C20 (after 20 days of diabetes)] |
| D | Mice blood samples before fasting / after fasting / [(D10 after 10 days of GSH treatment)/D20 (after 20 days of GSH treatment)] |

A: Control group, B: Diabetic GSH treated mice, C: Diabetic group, D: Normal GSH treated mice.

Treatment with reduced glutathione [9]

Preparing the doses considering 10 mg/kg human doses. The mice does will be 0.35 mg/mice normal antioxidant dose, the ten times dose were used to get the effective dose 11.37 mg/mice in 0.5 normal saline 0.9 % daily for B and D group IV through the tail in two periods 10 days and 20 days.

RNA isolation procedure

Isolation of total RNA was prepared according to Ambion procedure followed by [10].

Assay RNA yield [10]

The concentration of an RNA solution was determined by measuring its absorbance, by diluting an aliquot of the preparation in TE

(10 mM Tris-HCL pH 8, 1 mM EDTA) and reading the absorbance in a traditional spectrophotometer at 260 nm. To determine the RNA concentration in $\mu\text{g/ml}$, multiply the A_{260} by the dilution factor and extinction coefficient ($1 A_{260} = 40 \mu\text{g RNA/ml}$). $A_{260} \times \text{dilution factor} \times 40 = \mu\text{g RNA/ml}$.

Reverse transcription-polymerase chain reaction (RT-PCR) analysis [11]

Total RNA was obtained from adult testis, epididymus, spleen and pancreas, 1 μg of total RNA was used to synthesize cDNA using Invitrogen superscript III first-strand synthesis system for RT-PCR. In particular, (1 μg) of total RNA was combined with (5 μM) oligo (dT), (1 mM) dNTP mix, and diethyl pyrocarbonate-treated water in a final volume of (10 μl); the mix was incubated at (65°C) for (5 min.) and placed on ice for (1 min.) each sample was then added to (10 μl) of cDNA synthesis mix (2 μl) of 10x reverse transcriptase [RT] buffer, (4 μl of 25 mM) MgCl_2 , (2 μl of 0.1 M) dithiothreitol, (1 μl of 40 U/ μl) RNase OUT, and (1 μl of 200 U/ μl) superscript III RT and incubated at (50 °C) for (50 min.) the reaction was finally incubated at (85°C) for (5 min.) successively each PCR reaction was performed with (2 μl) of RT product to amplify cDNA fragments of mouse GR and GPX5. The PCR profile was (pre-denaturation at 94°C for 5 min followed by 35 cycles of denaturation at 94°C for 30 seconds, annealing at 62 °C for 30 seconds and extension at 72°C for 30 seconds, with an extra final extension at 72°C for 5 min.)

Primers for both enzymes GPx5 and GR were designed as follows Oligonucleotide primers used for GR amplification were:

Right: 5'-GGTGACCAGCTCCTCTGAAG-3' (Melting temp. 61°C)

Left: 5'-ACCACGAGGAAGACGAAATG-3' (Melting temp. 57°C)

The amplified region was of (161) bp.

Primers for GPX5 amplification were:

Right: 5'-CCGCAATAGGTAGCCACATT-3' (Melting temp. 57°C)

Left: 5'-AGGCCGAAAAGATGAAGAT-3' (Melting temp. 55°C)

The amplified region was (152) bp.

Gel electrophoreses [12]

The amplification products were then resolved by agarose gel electrophoresis and visualized by ethidium bromide staining. Images were captured with Polaroid film under ultraviolet light, as follows:

1. Agarose 0.8 % in TAE (1x) were melt in microwave
2. Cooled down at room temperature EtBr (0.5) $\mu\text{g/ml}$ was added and incubated in dark room temp. 30 min. in the tray
3. In jar of electrophoresis the DNA samples 10 μl each and ladder 6 μl were loaded
4. In 100 Voltage and for 50 min the course period of gel electrophoresis.
5. The gel was viewed in Ultra Violet light.

Glutathione assay [13; 14]

Extracting and assays done according to Sigma-Aldrich technical bulletin Glutathione Assay Kit/Catalog Number CS0260/ 2011 year catalogue.

Statistical Analysis[15]

The Statistical Analysis System- SAS (2004) was used to difference factors (group) in study parameters. The least significant difference (LSD) test at the comparative between means in this study, $P \leq 0.05$.

Results and Discussion

Blood sugar test results

In Table (2), the fasting blood sugar according to the age showed significant differences in which the lowest significant value checked for the control group while the highest significant fasting blood sugar recorded to (B20) group because this group relatively aged mice than the control group and the (D) group, These result recorded before inducing diabetes and without treatment with GSH to detect the differences in fasting level affected by age, The significantly highest sugar level fasting mice the relatively aged mice and the lowest value which were younger, Regarding the age the fasting blood level significantly differed [16], highly revealed this fact.

Table (2)

Effect of mice age on Fasting Blood sugar.

| group | Age /(days) | Fasting BT(mg/dl) |
|-----------------|-------------|------------------------|
| A | 196.60 | 68.30 ± 3.02 a |
| B ₁₀ | 321.50 | 110.00 ± 6.34 |
| B ₂₀ | 293.60 | 117.30 ± 6.98 b |
| C ₁₀ | 278.66 | 87.60 ± 4.78 |
| C ₂₀ | 172.00 | 105.33 ± 5.44 |
| D ₁₀ | 135.00 | 98.60 ± 5.50 |
| D ₂₀ | 142.00 | 71.00 ± 3.68 |
| LSD | ----- | 9.331 |

Number of mice for each group 6 males, values: mean± SEM, significant level: P≤0.05, **a**: the lowest significant value, **b**: the highest significant value A: control group, B₁₀: diabetic induced mice treated with GSH for 10 days, B₂₀: diabetic induced mice treated with GSH for 20 days, C₁₀: diabetic induced mice after 10 days diabetes, C₂₀: diabetic induced mice after 20 days of diabetes, D₁₀: healthy mice treated 10 days with GSH, D₂₀: healthy mice treated 20 days with GSH. **BT**: blood test (mg/dl).

Normal mouse will have blood glucose around 100 mg/dl (between 60-130) mg/dl [17] After a 4 hour fasting the (B₁₀, B₂₀, C₁₀ and C₂₀) show diabetic level blood sugar after induced with Alloxan intra peritoneal single dose injections those groups record a significant sugar level after treatment with GSH, that was obvious when comparing (B) with (C) in Table (3) and Fig.(1) for the levels of sugar reach their peak after 20 day of diabetes without any treatment as in (B) group which is completely decreased in sugar level reaching its healthy standers blood sugar level (D₁₀) reaches a fasting sugar level this is could be an indication that it should not be taken with a low blood sugar levels patients but with proceeding taking a moderate daily single dose it makes the sugar level maintained in its normal healthy levels as in (D₂₀) group. This result agree with [18]

Table (3)

Effect of fasting to the diabetic and normal mice with or without treatment with GSH.

| group | Fasting BT(mg/dl) | BT in (mg/dl) Diabetes /normal | Before IVF BT (mg/dl) |
|-----------------|------------------------|--------------------------------|-------------------------|
| A | 68.30 ± 3.02 a | 114.30 ± 6.61 a | 114.60 ± 6.28 |
| B ₁₀ | 110.00 ± 6.34 | 188.50 ± 16.75 | 139.00 ± 7.54 |
| B ₂₀ | 117.30 ± 6.98 b | 175.33 ± 14.54 | 140.60 ± 9.66 |
| C ₁₀ | 87.60 ± 4.78 | 152.60 ± 11.09 | 480.30 ± 22.67 b |
| C ₂₀ | 105.33 ± 5.44 | 277.33 ± 23.61 b | 425.66 ± 27.05 |
| D ₁₀ | 98.60 ± 5.50 | 146.30 ± 10.89 | 85.00 ± 4.91 a |
| D ₂₀ | 71.00 ± 3.68 | 136.30 ± 9.47 | 143.00 ± 7.47 |
| LSD | 9.331 | 14.754 | 43.277 |

Number of mice for each group 6 males, values: mean± SEM, significant level: P≤0.05, **a**: the lowest significant value, **b**: the highest significant value. A: control group, B₁₀: diabetic induced mice treated with GSH for 10 days, B₂₀: diabetic induced mice treated with GSH for 20 days, C₁₀: diabetic induced mice after 10 days diabetes, C₂₀: diabetic induced mice after 20 days of diabetes, D₁₀: healthy mice treated 10 days with GSH, D₂₀: healthy mice treated 20 days with GSH. **BT**: blood test (mg/dl).

The results in Fig.(1) showed the comparison between groups in the fasting and the normal status or the diabetic induced mice treated or not treated with glutathione daily injected for two different periods 10 and 20 days beside, this figure also showed the development of diabetes with time, the treatment with glutathione lowering the sugar levels even if it not reach the normal levels but it lowering the sugar level significantly than not treated group as in group (C) diabetic mice. And those results agreed with [19], at which their results concluded that Glutathione, critical antioxidant, supports insulin function, stabilizing blood sugar and helping to decrease sugar cravings and It is an important nutrient for maintaining optimal blood sugar levels, while in study represented by [17], the

decrease in biliary excretion of glutathione in diabetes may reflect an attempt to conserve glutathione by activation of the hepatic gamma-glutamyl cycle then they Leads to a conclusion that the disturbances of glutathione metabolism in diabetes are not typical of those seen in oxidative stress or food restriction. In turn leads to a conclusion that increasing GSH level in blood not decreasing sugar level.

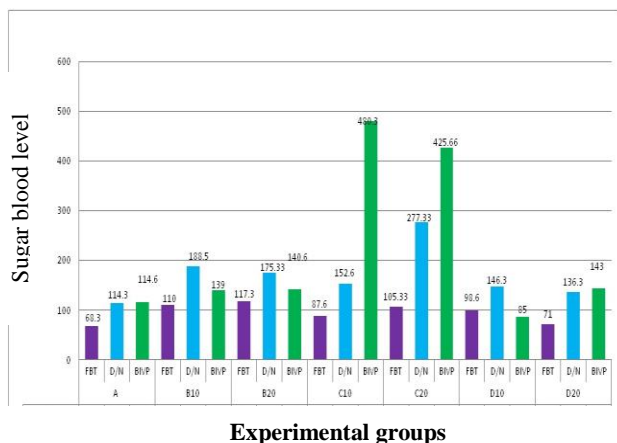


Fig. (1) Assessments of the experimental groups in mice blood sugar levels mg/dl, through diabetic or normal healthy status, and before in vitro fertilization, A: control group, B10: diabetic induced mice treated with GSH for 10 days, B20: diabetic induced mice treated with GSH for 20 days, C10: diabetic induced mice after 10 days diabetes, C20: diabetic induced mice after 20 days of diabetes, D10: healthy mice treated 10 days with GSH, D20: healthy mice treated 20 days with GSH, FBT fasting blood test, D/N: diabetic or normal mice blood test, BIVF before IVF blood test.

Glutathione assay study

In Table (4) the results of glutathione assay analyzed statistically in two dimensions horizontally between the GSH levels in different organs for the same group and the Fig. (2) revealed that epididymus have the significant higher levels of total glutathione than the other organs for control group, (B20) and (D20) this gives the evidence that the glutathione level in epididymus in healthy and treated mice with GSH gives the higher level in body organs, this result agreed with a conclusion done by [14] that the total glutathione assay take in account the reduced

and oxidized forms of glutathione in the epididymus epithelium and the sperms within the lumen rich with glutathione and glutathione enzymes GPx and GR, while in pancreas and testis record the lowest significant GSH levels in the body organs for groups (A, B20 and D20) in pancreas and the other groups in testis.

Table (4)
Glutathione concentration level in different organs between groups.

| Group | Testis | Epididymus | Spleen | Pancreas | LSD |
|-------|-----------------------|------------------------|----------------------|------------------------|-------|
| A | 0.21 ± 0.03 | 0.31 ± 0.06 b | 0.28 ± 0.06 | 0.19 ± 0.05 a | 0.069 |
| B10 | 0.30 ± 0.06 | 0.10 ± 0.02 a/c | 0.33 ± 0.06 b | 0.31 ± 0.06 | 0.061 |
| B20 | 0.23 ± 0.04 | 0.35 ± 0.06 | 0.29 ± 0.07 b | 0.21 ± 0.04 a | 0.043 |
| C10 | 0.10 ± 0.02 a | 0.19 ± 0.04 | 0.25 ± 0.04 b | 0.168 ± 0.04 c | 0.056 |
| C20 | 0.01 ± 0.0 a/c | 0.17 ± 0.04 b | 0.08 ± 0.01 c | 0.16 ± 0.03 c | 0.057 |
| D10 | 0.188 ± 0.04 a | 0.339 ± 0.05 | 0.21 ± 0.03 | 0.37 ± 0.05 b/d | 0.064 |
| D20 | 0.381 ± 0.06 d | 0.41 ± 0.07 b/d | 0.39 ± 0.06 d | 0.255 ± 0.05 a | 0.073 |
| LSD | 0.121 | 0.096 | 0.079 | 0.091 | --- |

Number of mice for each group 6 males, values: mean ± SEM, significant level: $P \leq 0.05$, **a**: the lowest significant value between organs, **c**: the lowest value between groups, **b**: the highest significant value between organs, **d**: the highest significant value between groups. A: control group, B10: diabetic induced mice treated with GSH for 10 days, B20: diabetic induced mice treated with GSH for 20 days, C10: diabetic induced mice after 10 days diabetes, C20: diabetic induced mice after 20 days of diabetes, D10: healthy mice treated 10 days with GSH, D20: healthy mice treated 20 days with GSH.

Looking at the results vertically between single organ compared in all groups the results revealed that (D) group records the significantly higher GSH activity between

other groups and the significantly lowest concentrations was recorded for all organs related to (C) group, however in Fig.(2) in general for the diabetic group (C) for all organs showed significant depletion in glutathione concentrations in all body organs and this agreed with the results that diabetes increase the oxidative stress as a result of antioxidant depletions [20].

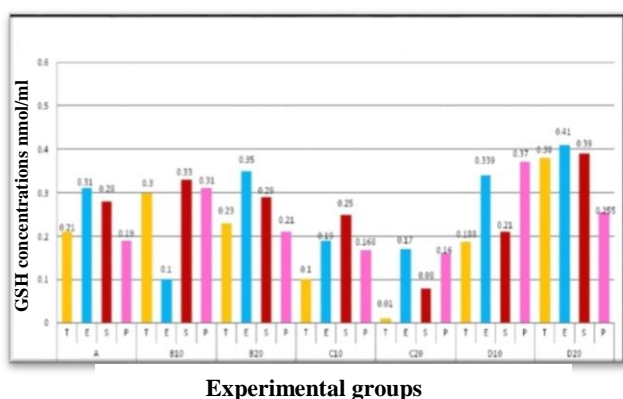


Fig. (2) Assessments of the experimental groups in the glutathione concentrations (nmoles/ml) in different body organs, A: control group, B₁₀: diabetic mice treated with GSH for 10 days, B₂₀: diabetic induced mice treated with GSH for 20 days, C₁₀: diabetic induced mice after 10 days diabetes, C₂₀: diabetic induced mice after 20 days of diabetes, D₁₀: healthy mice treated 10 days with GSH, D₂₀: healthy mice treated 20 days with GSH, T: testis, E: Epididymus, S: spleen, and P: pancreas.

And by increasing the level of the antioxidants supplements (glutathione) increase the level of the activity in the organs gradually. As started with B₁₀ till B₂₀ proceed in injecting the glutathione for diabetic mice increase significantly than diabetic (C) group the glutathione level reached the normal level as (A) group and epididymus still the highest organ in the body. These results agreed with a work done by [17] that referred that Glutathione is important in the regulation of the redox state, and a decline in its tissue level has often been considered to be indicative of increased oxidative stress in diabetes, but study represented by [20] showed the recycling antioxidant through the redox

system keep the Glutathione level naturally controlled.

Molecular study

Studying the expression of GPx and GR in different organs for diabetic mice with the 20 days diabetes the higher blood sugar level and poorer fertility outcomes compared with control group from isolating the total RNA then with the cDNA reverse transcript in a same concentration for each organs undergo RT-PCR, for the two redox system enzymes GPx and GR and through gel electrophoresis the bands could give a semi quantitative evaluation for the total RNA for the transcribed GPx and GR in pancreas, spleen, epididymus, and testis, the following gel electrophoresis revealed the resulted bands. According to [18] an increased expression of antioxidant enzymes was found to be positively associated with the free radical levels indicating that the body was trying to respond to the oxidative stress developed due to hyperglycemia during early phase. Hence concluded that the body tries to take care of the oxidative stress in the initial phase of the disease but this fails over a period of time leading to complications associated with diabetes. The results of Fig.(3) agreed with these conclusions, pancreas cDNA (2.684 $\mu\text{g}/\mu\text{l}$) highly expression revealed in bands for both GPx and GR of diabetic (C₂₀) group, Fig.(4) spleen cDNA (0.488 $\mu\text{g}/\mu\text{l}$) highly expression revealed in bands for both GPx of diabetic (C₂₀), Fig.(5) epididymal cDNA (0.5 $\mu\text{g}/\mu\text{l}$) highly expression revealed in bands for both GR for diabetic (C₂₀) group, Fig.(6) testis cDNA (2.196 $\mu\text{g}/\mu\text{l}$) highly expression revealed in bands for both GPx and GR for diabetic (C₂₀) group. While study done by [21] Disagreed with the experiment results when suggested that diabetes and oxidative stress decrease the natural antioxidants expression level in body organs.

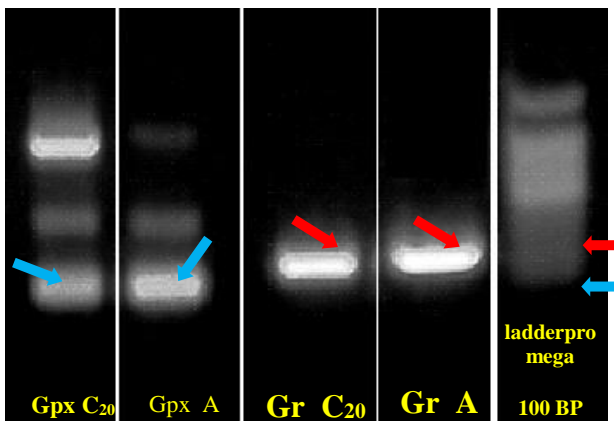


Fig.(3) Expression of glutathione peroxidase isoenzyme (GPX5) and glutathione reductase (GR) in the mice pancreas , a Reverse transcription–polymerase chain reaction (RT–PCR) amplification of GPX5 and GR transcripts using total pancreatic RNA obtained from mice of C20 group and control A group, Pancreas cDNA (from total RNA) concentration is (2.684 $\mu\text{g}/\mu\text{l}$) for each group. (Captured by the author) red arrow 161 bp, blue arrow 152 bp.

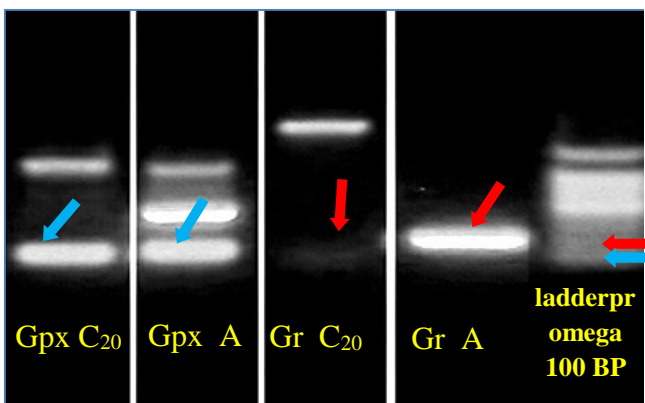


Fig. (4) Expression of glutathione peroxidase isoenzyme (GPX5) and glutathione reductase (GR) in the mice spleen, a Reverse transcription–polymerase chain reaction (RT–PCR) amplification of GPX5 and GR transcripts using total spleen RNA obtained from mice of C20 group and control A group, Spleen c DNA (from total RNA) concentration is (0.488 $\mu\text{g}/\mu\text{l}$) for each group, (Captured by the author) red arrow 161 bp, blue arrow 152 bp.

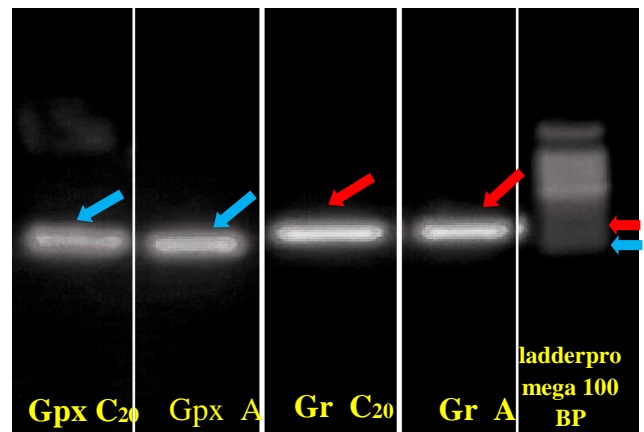


Fig. (5) Expression of glutathione peroxidase isoenzyme (GPX5) and glutathione reductase (GR) in the mice epididymus , a Reverse transcription–polymerase chain reaction (RT–PCR) amplification of GPX5 and GR transcripts using total epididymal RNA obtained from mice of C20 group and control A group, Epididymus c DNA (from total RNA) concentration is (0.5 $\mu\text{g}/\mu\text{l}$) for each group. (Captured by the author) red arrow 161 bp, blue arrow 152 bp.

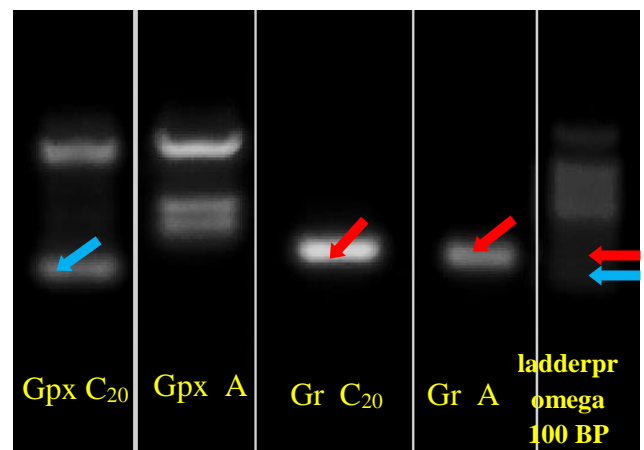


Fig.(6) Expression of glutathione peroxidase isoenzyme (GPX5) and glutathione reductase (GR) in the mice testis, a Reverse transcription–polymerase chain reaction (RT–PCR) amplification of GPX5 and GR transcripts using total testicular RNA obtained from mice of C20 group and control A group, testicular c DNA (from total RNA) concentration is (2.196 $\mu\text{g}/\mu\text{l}$) for each group, (Captured by the author) red arrow 161 bp, blue arrow 152 bp.

Conclusions

1. Total RNA isolation and RT-PCR study concluded that diabetes causes high oxidative stress in male mice which leads to increase expressions of antioxidants enzymes GR and GPx in pancreas, epididymus and testis.
2. The oxidative stress in diabetic male mice leads to depletion of free glutathione level in body organs (pancreas, spleen, epididymus and testis).
3. Diabetic high sugar level significantly decreased with GSH (11.37 mg/mice) daily IV injected in a period of 10 to 20 days in male mice.

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للاتزيمين GR و GPx وذلك كنتيجة لازيد الجهد الاكسدي الناتج بسبب الاصابة بداء السكري.

الخلاصة

درست تأثيرات الحقن وريديا بصورة يومية بالكولوتائون بتركيز (١١,٣٧ ملغم للفأر) على ذكور فئران صحية والفئران المصابة بالسكري المستحث بالالوكسان ومقارنة النتائج مع فئران صحية غير محقونة بالكولوتائون مجموعة السيطرة ومجموعة اخرى مصابة بداء السكري المستحث بالالوكسان ولفترتين ١٠ ايام و ٢٠ يوم. والدراسة كانت على هذه المجاميع الاختبارية بالنسبة الى فعالية دراسة التغيرات الحاصلة لنسب السكر بالدم اثناء الصيام وعند الاصابة بالسكري وبعد الحقن بالكولوتائون، ودراسة مستوى الكولوتائون الكلي في كافة المجاميع وللعضاء الطحال، البنكرياس، الخصية والبربخ واخيرا دراسة تعبير الجينات في الاعضاء السابق سردها بواسطة RT-PCR والترحيل الكهربائي بالهلام. اظهرت النتائج ان الحقن الوريدي اليومي بالكولوتائون للفترات عشرة ايام والعشرين يوم للذكور المصابة بالسكري، بالنسبة لقياسات نسب السكر بالدم فكانت الفئران المصابة بالسكري هي الاعلى نسب للسكر وقد تناقصت بصورة ملحوظة هذه المستويات بالحقن اليومي بالكولوتائون وللفترتين العشر والعشرين يوما، اضافة الي الجهد الاكسدي للفئران المصابة بداء السكري يؤدي الى انحدار تراكيز الكولوتائون الكلية لعضاء الجسم، واخيرا اجمالي RNA المستخلص من الاعضاء زيادة التعبير للجينات الخاصة