

The Role of Melatonin on GLUT-1 Expression in Rat Testicular Tissue Metabolism in Metabolic Syndrome

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ABSTRACT

Within the testis, glucose is essential for spermatogenesis. Melatonin cooperates with insulin in the regulation of glucose metabolism. Since the effects of melatonin on male reproductive system remain largely indefinite, we investigate the effects of melatonin on (GLUT-1), male hormonal level and oxidative state in testicular tissue in metabolic syndrome. Twenty-four rats had been divided randomly into three groups: control, fructose, and fructose plus melatonin. Metabolic syndrome (MS) was induced by fructose rich diet and melatonin was injected at a dose of 5 mg/kg dissolved in 1% ethanol in normal saline. After the end of the 6th-week of experimental period, body weight, testicular weight, and fat accretion were assessed. Serum lipid profile, glucose, insulin levels, insulin resistance, serum testosterone were measured. Rats were sacrificed by cervical decapitation, fresh testis were used; one for preparation of homogenate (GSH & MDA in testicular homogenate) the other testis underwent immunohistochemistry for GLUT-1 receptors. Fructose consumption significantly increased fasting glucose, fat accretion, serum lipids, insulin levels and insulin resistance, successful establishment of the MS model. Serum testosterone was significantly decreased compared to the control group. In addition, testicular MDA significantly increased and testicular GSH significantly decreased compared to the control group. GLUT-1 expression was increased compared to the control group. Melatonin supplementation significantly decreased fasting blood glucose, fat accretion, serum lipids, insulin levels and insulin resistance, compared to fructose group. Serum testosterone was significantly increased compared to the control group. In addition, testicular MDA significantly decreased and testicular GSH significantly increased compared to the control group. GLUT-1 expression was decreased compared to the control group. Conclusion: GLUT-1 expression may be concerned in glucose metabolism of testicular tissue in fructose induced MS. Melatonin protective effect may be linked to its antioxidant & lipid lowering effect with increased GLUT-1 expression.

Key Words: Melatonin; fructose; metabolic syndrome; insulin resistance; GLUT-1.

INTRODUCTION

A group of related metabolic abnormalities characterized the metabolic syndrome, including central obesity, hypertension, dyslipidemia, hyperglycemia, and

insulin resistance, while the causative factors were central obesity and insulin resistance [1] that is associated with increased risk of type 2 diabetes mellitus, cardiovascular diseases, and renal diseases [2].

Previous studies have shown that the fructose-fed rat model develops an insulin-resistance with a very similar metabolic profile to the human condition, including hyperinsulinemia, insulin resistance, hypertriglyceridemia, and decreased HDL cholesterol [3]. Concerning the regulation of energy homeostasis there are two hormones, insulin and leptin, which are not stimulated by fructose [4]. Therefore, fructose

How to Site This Article:

Sally S Donia and Dalia R Alsharaky. 2016. The role of melatonin on GLUT-1 expression in rat testicular tissue metabolism in metabolic syndrome. *Biolife*. 4(2), pp 347-356. doi:10.17812/blj.2016.4222
Published online: 9 June, 2016

consumption was associated with increased visceral obesity and inflammatory mediators [5].

Frequently, MS is associated with low testosterone level and sex hormone binding globulin (SHBG) levels in males [6], in comparison with age-corresponding subjects [7]. The hyperinsulinism may explain the increased inflammatory cytokines and increased waist circumference in these men [8]. The pathogenetic link between MS and male hypogonadism has not been completely clarified.

Proliferation and survival of cells relies on glucose uptake and its metabolism. It is carried out through glucose transporters. Two families of glucose transporters are known: the facilitated-diffusion glucose transporter family (GLUT family), and the Na⁺-dependent glucose transporter one (SGLT family). Successful transepithelial transport of sugars needs appropriate localization of glucose transporters [9].

Thirteen members of the family of facilitative sugar transporters (GLUT1-12 and the myo-inositol transporter HMIT) are recognized. These transporters have different substrate specificities, kinetic properties and tissue expression profiles [10]. The fertility of germ cells depends mainly on glucose metabolism and the spermatogenesis is disturbed in diabetes, causing infertility [11]. In sertoli cells, peritubular myoid cells, interstitial cells, early spermatocytes, and testicular blood vessels cellular uptake of glucose is mediated by GLUT 1, GLUT 2, and GLUT 3. This suggests that in cases of diabetes-associated disturbances GLUT 1, GLUT 2, and GLUT 3 are involved [12].

Melatonin (N-acetyl-5-methoxytryptamine) is a hormone mainly synthesized in the pineal gland of all mammals and is known for regulating the circadian rhythm [13]. Melatonin probably involved in glucose homeostasis [14]. As melatonin levels are known to be reduced in type 2 diabetic rats and humans. Also, pinealectomy of rats causes hyperinsulinemia [15]. So melatonin may play a role in metabolic disorders, such diabetes mellitus [16].

In photosensitive, seasonally breeding animals, melatonin can cooperate with the hypothalamic-pituitary axis influencing the release of gonadotrophins, which contribute in regulation of spermatogenesis, [17]. Melatonin also influences the quality of sperm [18] through modulation of testosterone production [19]. Due to its antioxidant capacity, melatonin proved to be efficient in protecting mammalian gametes and embryos during in vitro procedures [20]. Within the testis, the Sertoli cells (SCs) metabolism is sensitive to the action of hormones leading to spermatogenesis regulation [21]. These cells convert glucose to lactate, which is the main energy substrate for developing germ cells. GLUT1 and GLUT3 are the glucose transporters by which SCs take up glucose from the interstitial fluid [22].

The present study targets the protective effects of melatonin on the modulation of GLUT1 expressions in testicular tissue in fructose induced metabolic disorder.

MATERIAL AND METHODS

Animals and Experimental Design

This study was carried out in accordance with the regulations of Animal Experimentation Ethics Committee of faculty of medicine Menoufia University. Twenty-four adult male rats of Wister Albino weighing 120-150 g were used. The animals were housed at 20-24°C with a 12-h light, 12-h dark cycle and they were provided with standard rat chow and tap water freely available.

Twenty four rats were randomly divided into three groups (n = 8) as follows; Control group: Rats in this group received standard rodent diet. Fructose fed group: Rats in this group fed fructose rich diet (60% fructose (Technogene company, Egypt) mixed with standard rat chow) [23, 24]. Fructose plus melatonin treated group: Rats in this group fed fructose rich diet (60% fructose mixed with standard rat chow), and i.p. injected with melatonin (Bio Basic Inc, Canada) (5 mg/kg dissolved in 1% ethanol in normal saline), [24] daily for 6 weeks.

Melatonin solution was freshly prepared every day. Since ethanol was used as a melatonin's vehicle, control and fructose groups received 0.1% ethanol solution proportionately with body weight.

At the end of the experiment (after 6 weeks), body weight was assessed, animals were fasted overnight and blood samples were collected via retroorbital blood vessels. Blood samples were left for clotting for 10 min and centrifuged at 4000 rpm for another 10 min to isolate the serum and kept at -20°C for further analysis. Lastly, rats of all groups were sacrificed and the visceral, epididymal retroperitoneal fats and testicular weight were estimated. Both testes were rapidly excised, one testis was fixed 10% phosphate-buffered formalin solution, processed through paraffin embedding and prepared for immunohistochemical studies. The other testis was snap-frozen in liquid nitrogen and kept at -80°C. Testes were homogenized in ice-cold phosphate buffer (0.01 M, pH 7.4; 20% w/v) for various biochemical analyses.

Serum Biochemical Analysis

Lipid profile

Total cholesterol (mg/dl) and HDL-C (mg/dl) levels were determined following their hydrolysis and oxidation to yield colored quinoneimine derivatives using test reagent kits (Biodiagnostics, Egypt). Triglycerides (TGs) level (mg/dl) was estimated by a reagent kit (EMAPOL, Poland), in which TGs were hydrolyzed with lipoprotein lipase to form glycerol, which forms a complex with H₂O₂ giving a colored derivative. The obtained levels of total cholesterol, HDL and TGs were then used to calculate the serum level of LDL-C as that was described by Friedewald et al. [25].

LDL = Total Cholesterol - (HDL + Triglycerides/5).

Blood glucose

FBG level (mg/dl) is oxidized enzymatically to yield a red violet quinoneimine that can be determined colorimetrically using a test reagent kit (EMAPOL, Poland) [26].

Insulin levels:

Insulin level ($\mu\text{U/ml}$) was predicted following a solid phase two-site enzyme immunoassay (DRG Instruments GmbH, Germany) [27].

Homeostasis Model Assessment index (HOMA-IR)

Insulin resistance was evaluated by the HOMA-IR using the formula: $\text{Insulin } (\mu\text{U/ml}) \times \text{glucose (mg/dl)} / 405$ [28].

Testosterone level:

Serum testosterone concentrations were measured using enzyme-linked immunosorbent assay (ELISA) for testosterone (DRG, Marburg, Germany) [29].

Testicular glutathione (GSH) and malondialdehyde (MDA):

For the testicular content of GSH, a spectrophotometric kit (Biodiagnostic, Egypt) was used. In brief, the method is based on that the sulfhydryl component of GSH reacts with 5,5-dithio-bis-2-nitrobenzoic acid (Ellman's reagent) producing 5-thio-2-nitrobenzoic acid having a yellow color, that was measured colorimetrically at 405 nm (absorbance [sample] $\times 2.22/\text{g tissue used}$). Results were expressed as $\mu\text{mol/g tissue}$. The testicular content of lipid peroxides was determined by biochemical assessment of thiobarbituric acid reacting substance through spectrophotometric measurement of color at 535 nm, using 1,1,3,3-tetramethoxypropane as standard (absorbance [sample] $+0.004/0.0466$) as previously shown. The results were expressed as equivalents of MDA in testicular tissue homogenate in nmol/g tissue [30].

Immunohistochemical staining of Testis with GLUT-1:

Serial sections from mouse testis were dissected. Specimens were fixed in 10% formalin solution, and then it was sent to Pathology Department, Faculty of Medicine, Menoufia University, where it was submitted to routine tissue processing to be embedded in paraffin blocks. For each specimen sections of $4\mu\text{m}$ thickness were cut on routine slides for Hematoxylin and Eosin staining to assess the pathological changes, while sections for immuno-staining with GLUT-1 were cut on Poly L Lysine coated slides. The sections stained with H&E were examined under the light microscope for assessment of any pathological abnormality involving the seminiferous tubules, spermatogonia, sperms and the intervening interstitial tissue.

GLUT-1 purified rabbit polyclonal antibody (Cat. #RB-9052-R7) immunostaining was performed according to the supplier's protocol. It was received as

7.0 ml ready to use (Lab Vision Corporation 46360 Fermont Blvd. Fermont, CA 94538-6406, USA). Cutaneous squamous cell carcinoma tissue specimens were used as positive controls. Omission of the primary antibody served as a negative control.

Several sections were cut from the paraffin-embedded blocks with subsequent steps of deparaffinization and rehydration in xylene and graded series of alcohol, respectively. Antigen retrieval was performed by boiling in 10 ml citrate buffer (pH 6.0) for 20 min, followed by cooling at room temperature. The slides were incubated overnight at room temperature with purified rabbit polyclonal antibody to GLUT-1 (Cat. #RB-9052-R7) in humidity chamber. Finally, the detection of bound antibody was accomplished using a modified labeled avidin-biotin (LAB) reagent for 20 minutes then PBS wash. A 0.1% solution of diaminobenzidine (DAB) was used for 5 minutes as a chromogen. Slides were counter-stained with Mayer's hematoxylin for 5–10 minutes.

Interpretation of immunohistochemical results:

Any number of cells exhibiting membranous, cytoplasmic or both staining of GLUT-1 was considered positive in the studied cases and control specimens [31].

Testicular tissue in the three studied groups (control, fructose and fructose with melatonin) was assessed for:

1. Intensity of the stain: was graded as mild (+), moderate (++) or strong.(+++)
2. Staining pattern: cytoplasmic, membranous or membrano-cytoplasmic.
3. Expression Percentage: positive cells were counted and given a percentage over 200 cells of the whole section at 200X magnification in renal tissue [32].

For statistical purposes the percent of expression was further categorized into 3 groups:

- + : less than 30% GLUT-1 expression
 - ++ : from 30 to less than 60% GLUT-1 expression
 - +++ : more than 60% GLUT-1 expression
- 4- Distribution of GLUT-1: Patchy (irregular or not uniform distribution) or Diffuse (uniform distribution).

Statistical analysis:

The data were tabulated and analyzed by SPSS (statistical package for the social science software) using statistical package version 20 on IBM compatible computer. Quantitative data were expressed as mean \pm standard error of mean ($X \pm \text{S.E.M}$). Data from control and test groups were compared using one way ANOVA, followed by Turkey post Hoc test, Probability value of less than 0.05 was considered as statistically significant ($P < 0.05$).

RESULTS

Body Weight, Testicular weight and Fat Accretion

The body weight of fructose and fructose plus melatonin groups were found to be significantly higher ($P < 0.05$) than that of the control group. The testicular weight of fructose group was found to be significantly lower ($P < 0.05$) than that of the control group and fructose plus melatonin group was found to be significantly higher ($P < 0.05$) than that of the fructose-fed group. With regard to the major fat pad accretion, the fructose fed rats showed a significantly higher amount of visceral ($P < 0.05$), retroperitoneal ($P < 0.05$) and epididymal ($P < 0.001$) fat mass compared to their control counterparts indicating abdominal obesity, while fructose plus melatonin treated rats showed a significantly lower amount of visceral ($P < 0.05$), retroperitoneal ($P < 0.001$) and epididymal ($P < 0.001$) fat mass compared to fructose-fed rats (Table 1).

Table-1. Body weight, testicular weight and fat accretion (visceral fat, retroperitoneal fat and epididymal fat weights) (gm) in control, fructose fed and fructose plus melatonin treated groups.

| | Control | Fructose | Fructose & Melatonin |
|---------------------------------|-----------|------------|----------------------|
| Body weight (gm) | 207.5±3.2 | 275±3.1* | 286.25±2.8* |
| Testicular weight (gm) | 2.75±0.08 | 2.12±0.1* | 2.46±0.08# |
| Visceral fat (gm) | 2.87±0.1 | 4.06±0.08* | 2.91±0.18# |
| Retroperitoneal fat (gm) | 4.32±0.14 | 5.77±0.14* | 4.01±0.12# |
| Epididymal fat (gm) | 2.91±0.14 | 4.74±0.13* | 3.62±0.13# |

Data were expressed as mean ±S.E. (n=8). One way ANOVA: * $p < 0.05$, vs control; # $p < 0.05$, vs fructose group.

Serum biochemical analysis:

Serum lipid profile (cholesterol, TGs and LDL) in fructose group were significantly high ($p < 0.05$) compared to control group. In fructose plus melatonin group these parameters were significantly low ($p < 0.05$) compared to fructose group but still significantly high compared to control group. While HDL level in fructose and fructose plus melatonin groups was significantly low ($p < 0.05$) compared to control group (Table 2).

Blood glucose, serum insulin and HOMA-IR in fructose group were significantly high ($p < 0.05$) compared to their corresponding levels in control group, while these parameters in fructose plus melatonin group were significantly low ($p < 0.05$) compared to their corresponding levels in control group (Table 3).

Table-2. Serum lipid profile (cholesterol, TGs and LDL) and HDL level in control, fructose fed and fructose plus melatonin treated groups. Data were expressed as mean ±S.E. (n=8).

| | Control | Fructose | Fructose & Melatonin |
|------------------------------|------------|-------------|----------------------|
| Cholesterol (mg/dl) | 93.38±1.32 | 123.5±1.58* | 107.75±1.47*# |
| Triglycerides (mg/dl) | 72.62±1.17 | 95.62±1.22* | 84.25±1.5*# |
| HDL (mg/dl) | 44.87±1.49 | 35.12±1.14* | 37.25±1.51* |
| LDL (mg/dl) | 56.62±1.51 | 91.37±2.49* | 70.63±1.9*# |

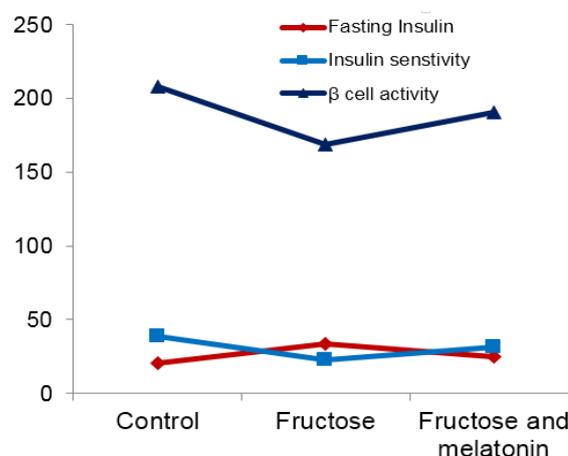
One-way ANOVA: * $p < 0.05$, vs control; # $p < 0.05$, vs fructose group.

Table-3. Blood glucose, serum insulin and HOMA-IR in control, fructose fed and fructose plus melatonin treated groups. Data were expressed as mean ±S.E. (n=8).

| | Control | Fructose | Fructose & Melatonin |
|------------------------------|------------|--------------|----------------------|
| Glucose level (mg/dl) | 86.12±2.74 | 113.61±1.25* | 95.12±1.66*# |
| Insulin level (µU/ml) | 20.62±0.59 | 33.62±0.94* | 24.75±0.59*# |
| HOMA-IR | 2.57±0.06 | 4.4±0.12* | 3.15±0.07# |

One way ANOVA: * $p < 0.05$, vs control; # $p < 0.05$, vs fructose group.

Figure-1: The effect of melatonin on fasting insulin, insulin sensitivity and beta-cell activity in fructose fed rats. Data are expressed as mean ±S.E.M. (n=8). * Sign when compared to control group, #Sign when compared to fructose-fed group.



Serum testosterone in fructose group was significantly low ($p < 0.05$) compared to their corresponding level in control group, while in fructose

plus melatonin group was significantly high ($p < 0.05$) compared to their corresponding level in fructose group (table 4). Testicular (GSH) in fructose group was significantly low ($p < 0.05$) compared to their corresponding levels in control group, while in fructose plus melatonin group was significantly high ($p < 0.05$) compared to their corresponding levels in fructose group (table 4). While testicular (MDA) in fructose group was significantly high ($p < 0.05$) compared to their corresponding levels in control group, while in fructose plus melatonin group was significantly low ($p < 0.05$) compared to their corresponding levels in fructose group (table 4).

Table-4: Testicular (MDA) testicular (GSH) and serum testosterone in control, fructose fed and fructose plus melatonin treated groups. Data were expressed as mean \pm S.E. (n=8). One way ANOVA: * $p < 0.05$, vs control; # $p < 0.05$, vs fructose group.

| | Control | Fructose | Fructose & Melatonin |
|---|-----------------|-------------------|----------------------|
| MDA (nmol/g.tissue) | 38.55 \pm 1.1 | 57.08 \pm 0.84* | 50.32 \pm 0.77*# |
| GSH (μmol/tissue) | 4.47 \pm 0.14 | 2.77 \pm 0.12* | 3.46 \pm 0.12*# |
| Serum testosterone (ng/ml) | 5.07 \pm 0.14 | 3.7 \pm 0.07* | 4.34 \pm 0.14*# |

GLUT-1 immunohistochemistry:

In a comparison done between the 3 groups, it was noticed that GLUT-1 was noticed to be absent in the control group, while the Fructose induced group came to express GLUT-1 in more than 60%, as for the group of fructose induced treated with melatonin it was noticed that the expression of GLUT-1 was decreased to less than 60% but not reaching to the control group.

Overexpression of Glut-1 was also noticed in the early spermatocytes and in the leydig cells in the fructose induced group while in the group treated with melatonin, the percent of expression decreased to less than 60% but not reaching to the percent of expression in the control group. On the other hand, the expression of GLUT-1 in the spermatids was noticed to decrease in the fructose induced in comparison with the control group.

GLUT-1 in the *fructose metabolic group* revealed heterogeneous expression, mainly in the spermatids together with spermatogonia and sertoli cells. GLUT-1 was expressed in the cytoplasm of the spermatids and sertoli cells (B) however; membranous expression of GLUT-1 was noticed in the early spermatocytes. Moderate GLUT1 expression was also seen in the Leydig cells (E).

Table-5: Comparison between the three studied groups regarding the percent of expression in the seminiferous tubules and the interstitial tissue of the testicular tissue.

| | Control group | Fructose induced group | Melatonin treated group |
|--------------------------------|---------------|------------------------|-------------------------|
| Sertoli cells | - | +++ | +++ |
| Early spermatocytes | ++ | +++ | ++ |
| Spermatids | +++ | ++ | +++ |
| Leydig cells | + | +++ | ++ |
| Peritubular myoid cells | ++ | ++ | ++ |
| Interstitial endothelial cells | ++ | ++ | ++ |

-:absent expression,+: less than 30% GLUT-1 expression, ++: from 30 to less than 60% GLUT-1 expression, +++: more than 60% GLUT-1 expression.

In fructose metabolic syndrome treated with *melatonin* GLUT-1 expression was mainly noticed in the Leydig cell in the interstitial tissue (C). As for the seminiferous tubules, GLUT-1 was mainly displayed in the spermatids and in the sertoli cells with focal expression in early spermatocytes (F).

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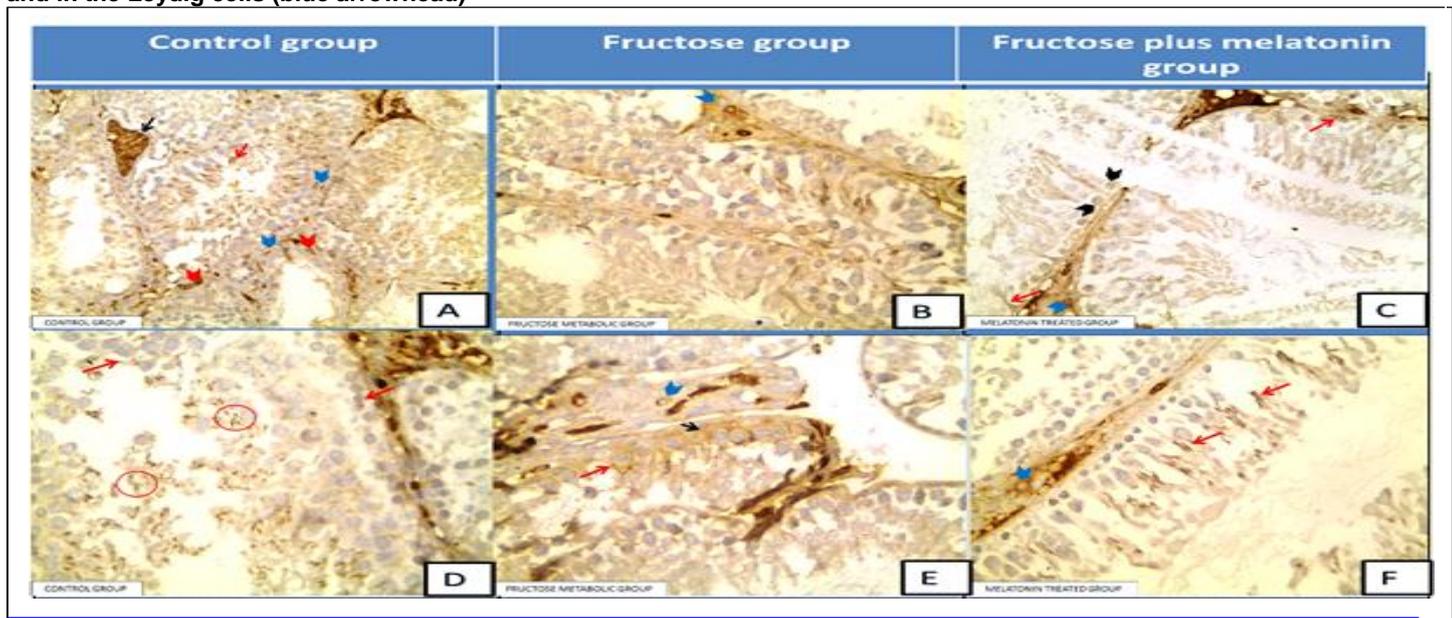
DISCUSSION

Melatonin hormone; is known as a circadian rhythm regulator; it has a combined metabolic regulatory effects and potent antioxidant activity [33], this promising multifunctional role of melatonin needs more researches. In this regard, the present study demonstrated that 5 mg/kg melatonin administered for 6 weeks improved body weight, blood glucose, the serum lipid profile, insulin resistance, serum testosterone, oxidative stress and expression of GLUT-1 receptors in testicular tissue in fructose fed rat model of metabolic syndrome.

Fructose intake is ineffective in stimulating insulin and leptin secretion, thus it may not induce a satiety response [34] resulting in increased food intake and

Figure-2: Immunohistochemistry staining of testicular GLUT-1. In *control group* the seminiferous tubules, focal patchy mild to moderate cytoplasmic staining of GLUT-1 was displayed in the spermatids both the late and early spermatids together with a focal mild expression in the spermatogonia. In the interstitial tissue, focal cytoplasmic expression of GLUT-1 was detected in the interstitial cells of Leydig and in the flat myoid cells. Intravascular RBC's stained with GLUT-1 was considered as an internal positive control (A). GLUT-1 is expressed in the elongated late spermatids while absent in the spermatogonia and sertoli cells (D). GLUT-1 in the *fructose metabolic group* revealed heterogeneous expression, mainly in the spermatids together with spermatogonia and sertoli cells. GLUT-1 was expressed in the cytoplasm of the spermatids and sertoli cells (B) however; membranous expression of GLUT-1 was noticed in the early spermatocytes. Moderate GLUT1 expression was also seen in the Leydig cells (E). In *fructose metabolic syndrome treated with melatonin* GLUT-1 expression was mainly noticed in the Leydig cell in the interstitial tissue (C). As for the seminiferous tubules, GLUT-1 was mainly displayed in the spermatids and in the sertoli cells with focal expression in early spermatocytes (F)

A): late spermatids (red arrow) , interstitial cells of Leydig (red arrowheads), flat myoid cells (blue arrowheads)and Intravascular RBC's (black arrow) B): sertoli cells (blue arrowhead) C): early spermatocytes (black arrowhead), sertoli cells (red arrow) and interstitial cells of Leydig (blue arrowhead)D): spermatids (red circles), spermatogonia (red arrows) E): spermatogonia (red arrow) sertoli cells (black arrow)and Leydig cells (blue arrowhead) F): spermatids (red arrows) and in the Leydig cells (blue arrowhead)



increased risk for obesity. Also, fructose may encourage food intake by hepatic ATP depletion [35] which occurs in animals and humans consuming it [36]. This was in agreement with our results, which showed significant increase in body weight and fat accretion in fructose group. Melatonin treatment in this study showed significant decrease in body weight and intra-abdominal adiposity, which was in agreement with Prunet-Marcassus et al., [37] who stated that in a model of overweight animals' melatonin may act as a regulator of body weight. On the other hand; the reduction in endogenous melatonin may alter metabolism, resulting in increased body weight and visceral adiposity [38].

In this study the fructose group showed significant increase in cholesterol, TGs and LDL, this was in agreement with Lê et al (2006) who stated that fructose has a lipogenic action and its consumption promotes the development of an atherogenic lipid profile [39]. Stimulation of hepatic LDL-triacylglycerol synthesis and secretion with decreased LDL-triacylglycerol clearance may be involved in this process [40], and contribute to hyperlipidemia and obesity [41]. On the other hand, melatonin administration showed significant decrease in

cholesterol, TGs and LDL. Hoyos et al. [42] showed that melatonin administration significantly reduce the increase in total cholesterol and LDL induced by a cholesterol-enriched diet and this hypolipidemic effects may be due to its ability to decrease absorption of cholesterol from intestine [43], inhibition of fatty acid transport, cholesterol biosynthesis and LDL-C accumulation [44].

The hallmark of metabolic syndrome is oxidative stress associated with a chronic low-grade inflammation; this oxidative stress results in impaired antioxidative-prooxidative balance which may contribute to the dysregulated secretion of adipokines that contributes to metabolic syndrome [45]. High fructose model of metabolic syndrome; was associated with overproduction of ROS [45] in this study it was evidenced by decreased testicular antioxidant defense mechanisms (GSH), as well as elevated testicular (MDA) that reflects the level of lipid peroxidation. On the other hand, melatonin administration significantly reduced the oxidative stress in the testicular tissue of rats with high fructose intake. She *et al.* showed that melatonin reduce oxidative stress, as its administration

was able to counterbalance a number of toxic reactants including ROS and free radicals [46]. It also increases the efficacy of classic antioxidants such as vitamin E, vitamin C and glutathione (GSH) [47]. In addition, several melatonin metabolites were themselves free radical scavengers [48]. This would suggest melatonin as a promising treatment in conditions associated with increased oxidative stress.

Our results pointed to an increased blood glucose level, insulin resistance (Homa-IR), decreased the pancreatic beta cell function and decreased testosterone level in the fructose fed group as compared to the control group. The reasons for the association between metabolic syndrome and decreased testosterone level are not completely investigated in this research. But according to Corrales et al (2006) the hyperinsulinemic state may lead to inhibition of the hepatic production of steroid hormone binding globulin (SHBG), with a resulting decrease in the total testosterone levels [49]. With low concentrations of testosterone there are higher levels of obesity and a centripetal distribution of body fat [6] consequently, there is an increased insulin resistance, which will generate a vicious cycle. The melatonin treated group showed significant decrease in blood glucose level, insulin resistance (Homa-IR), with increased pancreatic beta cell function and increased testosterone level; this was in agreement with Peschke & Muhlbauer [50]; who declared that melatonin has been involved in the regulation of insulin secretion and glucose/lipid metabolism. Zanquetta and his colleagues [51] showed that pinealectomized animals developed a state of glucose intolerance and increased insulin resistance. In mice maintained on a high-fat diet, melatonin administration markedly improved insulin sensitivity and glucose tolerance [52].

Melatonin has a significance role in preservation of male reproductive health; it increases testosterone synthesis either by decreasing the gonadotrophin-releasing hormone-induced luteinizing hormone (LH) secretion from the pituitary gland, or by direct action on Leydig cells [53]. Also, melatonin protects sperms from free radical damaging effect [54], and may have a role in testicular glycolytic metabolism [55]. Testicular glucose metabolisms are insulin-mediated [56] and melatonin may interact with insulin to exert that control.

Our results suggested that melatonin action on glucose uptake in testicular tissue is mainly mediated through modulation of GLUT1 expression that is also regulated by insulin. Expression of the GLUT1 in the control group of the rat testis is in concordance with Kokk et al., 2004 [57], reporting its expression in the peritubular myoid cells, macrophage like interstitial cells, testicular endothelial cells and spermatocytes. However, Kokk et al.'s study [12] reported absence of GLUT1 expression in the Sertoli cells or Leydig cells, which is in contrary to our results, in which GLUT1 was detected focally in the Leydig cells this expression of

GLUT1 in Leydig cells was previously reported by Chen et al., 2003 [58].

As for the distribution of GLUT1 receptor in control group, it is limited to the small area between the nucleus and the membrane, most probably the acrosomic system in the rounded early spermatids, is similar to the GLUT-8 expression in testis of mice as reported by Gomez et al., 2006 [59]. Also in the human sperms, GLUT1 is mainly localized in the acrosomal region, principle and end pieces of the tail Scheepers et al., 2004 [60].

Altered expression of GLUT1 in an experimentally induced pre diabetic state of the rats was reported by Rato et al., 2013 [55]. Meanwhile increased expression of GLUT1 was noticed in the prediabetic model rat induced by high-energy diet as reported by GL Ding et al., 2015 [61].

In the current study a decrease in the GLUT1 expression was noticed in the sertoli cells and leydig cells in melatonin treated group in comparison with high fructose group. Meanwhile the GLUT1 expression was increased in the melatonin treated group in comparison with the control group, our results are in concordance with [22] Rocha et al., in 2014 who reported significant increase in the GLUT1 level in sertoli cells cultured only with the melatonin.

Decreased expression of GLUT1 in the spermatids in fructose group in comparison with the control group, could be attributed to the effect of high glucose uptake which causes a decrease in luteinizing hormone (LH) and insulin receptor leading to decreased lactate production in Sertoli cells, which may retard germ cells development and slow down the process of spermatogenesis as proposed by Banerjee et al. in 2014 [62].

In melatonin group, it was noticed that there was mild increase in the GLUT1 expression in spermatids in comparison with fructose group, this increase could be attributed to the increased acetate production in sertoli cells by the melatonin. Acetate can be used by sertoli cells as a source/store of energy and thus may improve the fertility of diabetic models as reported by Rocha et al., in 2014 [22].

Conclusion

Several studies have demonstrated the potential beneficial actions of melatonin in the MS. Apart from its antioxidant actions, the metabolic actions of melatonin should be considered in MS. Melatonin may improve the reproductive function of rats having MS, and its action on glucose uptake in testicular tissue is mainly mediated through modulation of GLUT1 expression, further studies are needed to clarify the role of this hormone in male reproductive health in general.

Conflict of Interests

Authors declare that there is no conflict of interests regarding the publication of this paper.

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