Role of serotonin on pituitary-thyroid functions and antioxidants in diabetic male rats

Fouad Ziedan Hamzah¹  Sumayah Faruq Kasim²

¹Faculty of Veterinary Medicine, University of Kufa, Iraq.
²College of Health and Medical Techniques, Middle Technical University, Iraq.

Corresponding author: SFK, e-mail: sumayah.faruq@mtu.edu.iq; ORCID ID: 0000-0003-1809-6832
Co-author: FZH, e-mail: fouadz.alquraishi@uokufa.edu.iq; ORCID ID: 0000-0003-0642-3381

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Abstract
Diabetes mellitus causes an increase in oxidative stress. Serotonin is a hormone its level in circulation reduced in diabetes; recently, it has been found to possess antioxidant qualities. The purpose of the study was to look at the impact of diabetes on pituitary-thyroid functioning, and serotonin role in modulating these effects in a streptozotocin-induced diabetes mellitus rat model. The thirty-two healthy male rats with a body weight of 180-200 gm were split equally into 4 groups, group [1]: control, group [2]: Serotonin, group [3]: diabetic, and group [4]: Serotonin-diabetic. The rats were weighed and serum levels of glucose, insulin, malondialdehyde, superoxide dismutase, catalase, glutathione peroxidase, serotonin, thyroid stimulating hormone, thyroxine, and triiodothyronine were measured. Our results presented that there was a significant increase in T3, T4, glucose, and MDA in the diabetic group compared to the control, while a significant decrease in T3, and T4, glucose, and MDA in the Serotonin with diabetic group compared to diabetic group. Furthermore, a significant decrease in TSH, serotonin, insulin and GPX, CAT, and SOD antioxidants in the diabetic group compared to control, while a significant increase in serum TSH, serotonin, insulin and CAT, GPX and SOD antioxidants in the Serotonin+diabetic group compared to diabetic group as well as a significant increase in serum insulin in the Serotonin group compared to all other groups. Serotonin plays a protective role against diabetes-induced and pituitary-thyroid disorder caused by diabetes which might be because of its antioxidant qualities and ability to maintain glucose and insulin balance.

Keywords: Anti-oxidants, Diabetes Mellitus, Oxidative stress, Pituitary-thyroid, Serotonin.
Different functions of 5-hydroxytryptamine are found in the non-neuronal and neuronal mechanisms. It functions like a neurotransmitter, a mitogen, and a hormone. Serotonin was given this name just after Latin word serum as well as the Greek word tonic since it was assumed to be a vessel-constrictor contained in platelets at the period of its discovery (1918) [4, 5]. The physiological consequences of 5-hydroxytryptamine are initiated through its absorption inside the cells by 5-hydroxytryptamine transporter (5-HT) and successfully completed by membrane-bound 5-hydroxytryptamine transporter receptors [6].

Serotonin is a biologically active monoamine neurotransmitter in which is produced centrally inside neurons from L-tryptophan in considerable quantities in neurons of the central nervous system (CNS) and peripherally in platelet granules in the gastrointestinal tract (GIT) [7]. Serotonin regulates a variety of vascular dynamics, including vascular resistance, blood pressure management, and platelet aggregation [8]. Seven distinct types of serotonin receptors (5-HT1 to 5-HT7), which are dispersed throughout the body, and mediate its activity [9].

Following a 5-hydroxytryptophan preload, a precursor of serotonin, it has long been recognized that 5-HT and insulin co-localize in the same vesicle in pancreatic β-cells. Additionally, serotonin has been connected to the control of glucose levels [10, 11]. 5-HT has recently been discovered to be a placental lactogen's downstream molecule, regulating how β-cells adapt to pregnancy [12]. Tph1 expression in pancreatic β-cells increases dramatically as a result of lactogens released throughout the pregnancy, which causes a large synthesis of serotonin in β-cells [12]. Through the 5-HT3 receptor, islet serotonin improves beta cell glucose responsiveness, leading to greater islet glucose-stimulated insulin secretion (GSIS) [13, 14]. Additionally, mid-gestation is when the expression of the 5-HT2B receptor rises to encourage β-cell proliferation and increase β-cell mass, whereas the 5-HT 1D receptor expression rises near the end of a pregnancy to decrease β-cell mass [12].

In dietary insulin resistance, 5-HT regulates insulin secretion as well. In response to an HFD, glucose intolerance was observed in -cell-specific Tryptophan hydroxylase 1 gene (Tph1) Knock-Out (KO) and 5-Hydroxytryptamine Receptor 3A (Htr3a) Knock-Out (KO) mice, but not in a wild-type genotype (WT) mice. The Glucose-stimulated insulin secretion (GSIS) was disrupted in islets extracted from high-fat diet (HFD-fed) Htr3a KO and -cell-specific Tph1 KO mice, and 5-HT administration increased insulin production from the -cells of the latter animals but not the Htr3a KO islets [13]. Additionally, the embryonic pancreas has been found to produce 5-HT. Particularly, at the time just after birth, when β-cells are actively multiplying, Tryptophan hydroxylase 1 gene expression is increased in newborn β-cells [15]. Since 5-HT is produced by -cells during the perinatal period and during pregnancy, two physiological conditions that promote –cell proliferation, it is thought that 5-HT is a critical controller of –cell proliferation and insulin release [12-14, 16].

The purpose of this research was to look at diabetes mellitus' effects on the pituitary-thyroid functioning and the function of serotonin in modulating these results in a streptozotocin-induced diabetes mellitus rat model.

MATERIALS AND METHODS
Animal Ethical Approval:
This study was approved by the ethics committee of the Faculty of Veterinary Medicine, University of Kufa and conforms to the Guide for the Care and Use of Laboratory Animals (No.9129).

Sampling:
Thirty-two healthy albino male adult rats weighing 180–200 g were used in this investigation; they were bought from the animal house at the Faculty of Veterinary Medicine at the University of
Kufa. Rats were housed at physiology animal house at Faculty of Medicine - University of Kufa in four-per-cage steel wire cages under sanitary conditions [17-19]. Before the trial began, rats spent one week getting used to their new surroundings. They were kept at ambient temperature, a 12-hour light/dark cycle maintained, and unrestrained access to water. Rats were acclimated to their new surroundings for one week prior to the experiment.

Rats were split into four equal groups at random. In group 1, the control group, each rat received a single intraperitoneal dose of 0.5 mL of saline for 10 days. In group 2, each rat received a single intraperitoneal dose of freshly synthesized serotonin hydrochloride [Sigma Aldrich Co., USA powder] at a quantity of 1.6 mg/kg of body weight (b.w) dissolved in saline for 10 days [20], group 3: diabetic group, which was given an intraperitoneal injections of fresh manufactured streptozotocin [Sigma Aldrich Company, USA] at a dose of 60 mg/kg diluted in saline to develop experimental diabetes [21] and overnight the fasted rats. The fasting glucose level was measured using a glucometer after 72 hours [ACCUCHEK, Roche Diagnostics, Germany], this study included rats with blood glucose levels more than 360 mg/dl. [22], group [4]: Serotonin-treated diabetic rats received a single 1.6 mg/kg intraperitoneal injection of serotonin of [b.w] daily for 10 days.

Biochemical Tests:
Serum levels of glucose: So according Tietz, (1995) [23], it is tested using a 546 nm spectrophotometer (Spectronic 3000 Array, Germany) and glucose enzymatic (GOD-PAP) - liquizyme Kits from Biotechnology, Egypt.

Statistical analysis:
The data underwent an analysis of variance, and the significant differences at P≤0.05 were assessed by ANOVA, one-way by utilizing the statistical software’s sigma statistical.

RESULTS
Effect of Serotonin on T3, T4, TSH and Serotonin levels showed in Table (1) which demonstrates that, in comparison to the control group, the diabetic group experienced a significant (P≤0.05) increase in serum T3 and T4, whereas the Sero with diabetic group experienced a significant (P≤0.05) decrease in serum T3 and T4 in comparison to the diabetic group. However, there was a substantial (P≤0.05) drop in serum TSH and serotonin in the diabetic group when compared to the control group and a significant (P≤0.05) rise in serum TSH and serotonin in the Sero with diabetic group when compared to the diabetic group.
Table (1): Effect of Serotonin on T3, T4, TSH and Serotonin levels in diabetic adult male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>T3 ng/ml</th>
<th>T4 μlU/ml</th>
<th>TSH μlU/ml</th>
<th>Sero ng/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>2.058±0.027 b</td>
<td>7.104±0.048 d</td>
<td>1.078±0.100 b</td>
<td>7.106±0.045 b</td>
</tr>
<tr>
<td>Sero+diabetic</td>
<td>2.084±0.042 b</td>
<td>8.074±0.089 c</td>
<td>1.260±0.426 b</td>
<td>9.638±0.316 a</td>
</tr>
<tr>
<td>Sero</td>
<td>1.516±0.219 c</td>
<td>9.072±0.050 b</td>
<td>1.934±0.449 a</td>
<td>9.290±0.430 a</td>
</tr>
<tr>
<td>diabetic</td>
<td>4.052±0.042 a</td>
<td>10.316±0.071 a</td>
<td>0.500±0.141 c</td>
<td>4.130±0.136 c</td>
</tr>
<tr>
<td>LSD</td>
<td>0.165</td>
<td>0.094</td>
<td>0.430</td>
<td>0.369</td>
</tr>
</tbody>
</table>

Significant group differences are indicated by tiny letters at [p≤0.05] level.

Effect of Serotonin on Glucose and Insulin levels presented in Table (2) which demonstrates that, the diabetic group had a substantial (P≤0.05) rise in glucose compared to the control group, whereas the Sero with diabetic group had a significant (P≤0.05) drop in glucose compared to the diabetic group. While there was a significant (P≤0.05) decrease in serum insulin in the diabetic group when compared to the control group, there was also a significant (P≤0.05) increase in serum insulin in the Sero with diabetic group when compared to the diabetic group, as well as a significant (P≤0.05) increase in serum insulin in the Sero group when compared to all other groups.

Table (2): Effect of Serotonin on Glucose and Insulin levels in diabetic adult male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>Glucose mg/dl</th>
<th>Insulin μlU/ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>85.188±1.959 c</td>
<td>27.540±0.359 b</td>
</tr>
<tr>
<td>Sero+diabetic</td>
<td>165.538±0.802 b</td>
<td>18.52±0.297 c</td>
</tr>
<tr>
<td>Sero</td>
<td>80.126±0.026 d</td>
<td>29.322±0.393 a</td>
</tr>
<tr>
<td>diabetic</td>
<td>579.656±4.855 a</td>
<td>8.116±0.063 d</td>
</tr>
<tr>
<td>LSD</td>
<td>3.501</td>
<td>0.420</td>
</tr>
</tbody>
</table>

Significant group differences are indicated by tiny letters at [p≤0.05] level.

Finally, the effect of Serotonin on CAT, GPX, SOD antioxidants and MDA levels illustrated in Table (3) which demonstrates that MDA significantly increased in the diabetic group (P≤0.05) when compared to the control group, but significantly decreased in the Sero with diabetic group (P≤0.05) when compared to the diabetic group and the control group. On the other hand, the diabetic group had a substantial (P≤0.05) drop in CAT, GPX, and SOD antioxidants compared to the control group, whereas the Sero with diabetic group had a significant (P≤0.05) increase in all antioxidants GPX, CAT and SOD compared to the diabetic group.

Table (3): Effect of Serotonin on CAT, GPX, SOD antioxidants and MDA levels in diabetic adult male rats

<table>
<thead>
<tr>
<th>Parameters</th>
<th>CAT U/ml</th>
<th>GPX μmol/L</th>
<th>MDA nmol/L</th>
<th>SOD ng/Ml</th>
</tr>
</thead>
<tbody>
<tr>
<td>Control</td>
<td>758.25±26.925 c</td>
<td>91.202±1.170 b</td>
<td>4.108±0.134 c</td>
<td>7.036±0.228 a</td>
</tr>
<tr>
<td>Sero+diabetic</td>
<td>911.61±5.090 a</td>
<td>92.148±1.287 b</td>
<td>5.538±0.360 b</td>
<td>6.026±0.392 b</td>
</tr>
<tr>
<td>Sero</td>
<td>876.04±13.627 b</td>
<td>102.448±0.979 a</td>
<td>3.136±0.136 d</td>
<td>7.152±0.229 a</td>
</tr>
<tr>
<td>diabetic</td>
<td>349.32±23.534 d</td>
<td>65.584±0.852 c</td>
<td>9.058±0.165 a</td>
<td>3.428±0.451 c</td>
</tr>
<tr>
<td>LSD</td>
<td>26.963</td>
<td>1.598</td>
<td>0.322</td>
<td>0.471</td>
</tr>
</tbody>
</table>

Significant group differences are indicated by tiny letters at [p≤0.05] level.
DISCUSSION

The most common metabolic illness, diabetes, affects people from all socioeconomic backgrounds. Reduced insulin release from the pancreatic β-cells or diminished insulin activity on its target organs may both contribute to the condition. Therefore, comprehensive knowledge of the control of insulin secretion is necessary to comprehend how to prevent and cure diabetes. Serotonin is a hormone that we detect in our study as an improver to modulate the release of insulin which in turn regulates the levels of glucose in the blood. To have a better knowledge of type 1 and type 2 diabetes mellitus causes and the young-onset maturity of diabetes, several animal models have been developed [25–27]. Our findings showed a significant increase in serum glucose levels in the STZ-diabetic group as compared to the control group, while a significant decrease in serum glucose levels in the Sero with diabetic group as compared to STZ-diabetic group. However, a significant decrease in serum insulin was observed in the STZ-diabetic group when compared to the control group, while significant increases in serum insulin were observed in the Sero diabetic group when compared to STZ-diabetic group as well as in the Sero group when compared to all other groups. These findings obviously show the ameliorative role of serotonin hormone on insulin and glucose levels in diabetic rats. As described in the introduction part, mounting evidence suggests that 5-HT has a beneficial impact on the body's overall glucose homeostasis. However, many regulatory features of insulin secretion are unclear because of the intricate machinery involved. The function of serotonin in β-cells is one topic that has baffled scientists for more than three decades [28, 29]. Within β-cells, 5-HT is synthesized [30], it is kept in their secretory β-granules alongside with insulin [11], and when pancreatic islets are activated, it is also co-secreted with glucose [31, 32].

Prior research has shown a unique mode of serotonin activity in blood platelets that is dependent on the hormone's long-term covalent attachment to signaling proteins, or "serotonylation." The researchers found similar process in pancreatic beta cells as well. Serotonylation controls the release of storage granules from these cells, just like it does in thrombocytes. Serotonin regulates insulin secretion, which is the most significant hormone in controlling blood glucose levels in both humans and animals, under normal circumstances. In addition to the study's significance to the knowledge of serotonin's function in the common illness diabetes, the unique example of serotonylation serves as an illustrative illustration of the physiological significance of protein monoaominylation in general. Similar effects can be achieved by other monoaminergic hormones such as histamine, dopamine, and norepinephrine. Monoaminylation, like protein phosphorylation, has a significant influence on a number of biological processes, and Berlin-based researchers have embraced the challenge to identify them. The findings, which were reported by researchers from Berlin, provide an explanation for what serotonin is doing in the pancreatic beta cells and broaden our comprehension of the function of protein monoaminylation in physiological processes that are important for illness [33].

In this study we focused our interest in the relation between pituitary-thyroid function, antioxidants and serotonin; this is due to the lack of previous studies on this aspect. Furthermore, our data presented that as compared to the control group, the STZ-diabetic group showed a substantial increase in blood T3, T4, and MDA and a significant decrease in serum TSH, serotonin, and CAT, GPX, and SOD antioxidants. The Sero with diabetic group showed a significant increase in serum T3, T4, and MDA, as well as a significant increase in serum TSH, serotonin, and CAT, GPX, and SOD antioxidants when
Several scientists and researchers have found that there is a dual relationship between the thyroid gland and diabetes, a disease caused by insulin resistance in the body, and insulin is a hormone responsible for controlling blood sugar; it proven a direct relationship between hypothyroidism and diabetes and vice versa, high blood sugar by increasing the amount of sugar secreted by the liver and/or increasing the body's resistance to insulin can lead to high levels of thyroid hormones. Despite decades of research, much is still unknown about serotonin, despite the fact that it affects many aspects of health and has a wide range of effects. Apparently, thyrocytes [the cells that make up thyroid] can be stimulated into action by serotonin, as stated by Dobberstein [34], it stimulates thyroid cell proliferation and apoptosis, this suppose aligned with our findings in improving T3 and T4 levels as a result of the serotonin effect in ameliorating the oxidative stress resulting from diabetes on thyroid cells.

Numerous physiopathological conditions and degenerative illnesses are linked to the oxidation of biological components including DNA, proteins, or lipids [35-38].

These research results taken together compelled us to describe the ways in which 5-HT exerts its antioxidant capabilities. Numerous experimental investigations have demonstrated the important antioxidant effects of 5-HT. The molecule's chemical system has produced strong free radical scavenging characteristics in vitro [39-42]. Additionally, 5-HT has been utilized to stop the production of free radicals caused by chemicals in vitro [43].

We assumed that the 5-HT has a modulating effects on T3, T4, CAT, GPX and SOD antioxidants, results clearly show 5-HT efficiency, suggesting its direct involvement in modulating the effects of diabetes mellitus in rats model induced by streptozotocin as well.

CONCLUSION
Serotonin may have a preventive role against diabetes-induced pituitary-thyroid disturbances because of its antioxidant characteristics and maintaining insulin and glucose concentrations.

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CONFLICT OF INTEREST
There is no conflict of interest.

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