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Reversal in Thyroid Gland Impairments by Exogenous Melatonin in Streptozotocin Induced Diabetic Male Rat Model

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ABSTRACT: Present study was designed to demonstrate the effect of exogenous melatonin (MEL) against the streptozotocin (STZ) induced alterations in thyroid gland in rat model. STZ was injected at a dose of 15mg/kg for consecutive six days to induce diabetes. Twenty four male rats were randomly divided into four different groups, each group containing 6 rats. The blood glucose was assessed after 72 hours of STZ injection and was continuously repeated upto 7th day to confirm the induction of diabetes, animals with blood glucose level 250mg/kg were considered as diabetic and were further divided into different experimental groups. After 24 hours of the last treatment of melatonin, all the animals were weighed and partially anaesthetized with diethyl ether. Thyroid gland was dissected out some portion fixed in Bouin's fixative and rest was kept for tissue biochemical analysis. In STZ induced diabetic rats LPO level was significantly higher, while as total protein content, GSH and T3, T4 and TSH level revealed significant decrease. However, melatonin administration significantly restored the biochemical and hormonal alteration towards the control level. The biochemical and hormonal studies were supported by histopathological investigation, in which follicular population was found decreased in STZ diabetic rats, which was restrained by melatonin administration. Hence, on the basis of the present findings it might be concluded that melatonin might be used to modulate thyroid gland functions.

I. INTRODUCTION

Diabetes is an endocrinological disorder resulting due to reduction in insulin secretion, insulin sensitivity, or both which in turn leads to chronic hyperglycemic condition with disturbances of carbohydrate, protein and fat metabolism [1]. It covers a wide range of heterogeneous diseases such as tissue or vascular damage leading to severe diabetic complications such as retinopathy [2], neuropathy [3,4], nephropathy [5,6], cardiovascular complications [7] and ulceration [8] along with some alterations in other endocrine glands. Diabetes mellitus and thyroid diseases are two common endocrinopathies found in the adults. Because both insulin and thyroid hormones contributes in cellular metabolism, low or excess levels results in functional aberrations [9,10].

Thyroid gland is involved in the metabolic homeostasis of the body. T3 and T4 influence reproduction, growth and differentiation and metabolism. Thyroid hormones regulate many physiological activities such as carbohydrate and lipid metabolism, oxygen consumption [11], increased rate of absorption from the gastrointestinal tract and even enhance the insulin secretion [12].

Diabetes appears to influence thyroid functions at two sites: first at the level of hypothalamic control of TSH release and secondly at the peripheral thyroid level [13]. During hyperglycemic condition concentration of T3 and T4 level decreases in serum but at the same time increases the level of rT3 [13,14]. The previous studies reported that serum TSH level decreases and reduced response to TRH observed during diabetes [15]. Hyperglycemic induces an increase in the generation of free radical [16]. These free radical reactive species reacts with the lipid molecules of cell membrane and lead to cell membrane disintegration and cell membrane loses its property of permeability and intracellular contents leak out from the cells into blood circulation [6]. Therefore, the normal functioning of the cell becomes disturbed. Mitochondria, rough endoplasmic reticulum degenerates and Intracolloidal as well as intraepithelial thyroglobulin are reduced. Diabetes induces different structural impairments in thyroid follicle such as epithelial cells becomes flattened, RER cisternae becomes empty and degeneration of mitochondria. Such alterations in cellular functions ultimately affect the intracellular environment and finally lead to the cell death.

Melatonin is anindoleamine secreted by pineal gland of vertebrates [17]. It has been reported that melatonin is also synthesized from some non-pineal tissues such as parafollicular cells of thyroid [18]. Melatonin has important role apoptosis, antioxidative [6,19], anti-inflammatory, oncostatic [20], neuroprotective [4] mitochondrial homeostasis [21], anti-aging, antiproliferative or immunomodulatory activities [20]. Besides its other regulatory actions, it also regulates thyroid growth and secretory processes. Melatonin mediate its actions through some specific membrane bound receptors, MT1 and MT2 [22] as well as ROR/RZR retinoid receptor [23] present on follicular and parafollicular cells of thyroid [24] and also plays a crucial role in the regulation of circulating thyroxin hormones.

Melatonin was discovered to be a free radical scavenger that scavenges hydrogen peroxide (H_2O_2), hydroxyl radical (OH), nitric oxide (NO), peroxynitrite anion (ONOO-), hypocholorous acid (HOCl), singlet oxygen (O^{2-}), superoxide anion (O^{2-}) and peroxyl radical (LOO), independent of receptor mediated effects. MEL stimulates a number of antioxidative enzymes, including glutathione reductase, glutathione peroxidase etc. [25], and prevents the tissues from oxidative damage. MEL itself interacts with free radicals to form stable end-products [26]. It increases the efficiency of the electron transport chain and, as a consequence, to reduce electron leakage and the generation of free radicals. It has an important role in protecting thyroid follicular cells against oxidative damages [27]. Therefore, present study has been designed to demonstrate the effect of exogenous melatonin treatment in STZ induced thyroid gland in rats.

II. MATERIALS AND METHODS

A. Chemicals, reagents and instruments

Streptozotocin, Melatonin, Citrate monohydrate, Sodium citrate Thiobarbituric acid (TBA), Tris-hydrochloric acid (Tris-HCl), Phosphoric acid and Butylated Hydroxy Toluene (BHT), Glutathione reduced (GSH), Glacial acetic acid, Dithio-bis 2-nitrobenzoic acid (DTNB), Nicotine Adenine Dinucleotide Phosphate (NADPH). All the chemicals and reagents were of analytical grade purchased from Sigma Aldrich and Himedia. ELISA kits for hormonal assays (T3, T4 and TSH) were purchased from Biogennix, India. UV-visible spectrophotometer of Perkin Elmer, Centrifuge (Remi), ELISA Reader (TECKEN).

B. Animal maintenance and experiment

Rats of Wistar strain weighing approximately 175±10 g of same age group were acquired from Defence Research and Development Establishment (DRDE) Gwalior, M.P. India and were housed in plastic cages under 12h light/dark cycle at 25±2°C temperature and 60-70% humidity. Rats were given access to pellet diets and water *ad libitum.*

All experimental procedures were approved by the instu tional animal ethical committee, Guru Ghasidas, Vishw avidyalaya (Central University), Bilaspur, Chhattisgarh, India (Registration No: 994/Go/ERe/S/06/CPCSE).

Rats were acclimatized for one week and were grouped into following experimental groups:

III. EXPERIMENTAL DESIGN

Group I: Normal control rats
Group II: Diabetic control (STZ 15mg/kg b. w. 6 days, i.p)
Group III: STZ +MEL [STZ 15mg/kg (6days) + 1mg/kg b. w. 4 weeks]
Group IV: MEL (1mg/kg b. w. 4 weeks)

A. Induction and assessment of diabetes

Streptozotocin (STZ) 15 mg/kg was prepared in 0.1m citrate buffer having pH 7.4 and was injected intraperitoneal route for six days. STZ was given for six days continuously through intraperitoneal route and after 72 hours blood glucose level of STZ administered rats were checked with the help of semiautomatic glucometer (ACCU CHECK ACTIVE). Rats having blood glucose level greater than 250mg/dl were confirmed as diabetic model. After successful induction animals were divided into four different experimental groups as under the below given experimental design. Animals were sacrificed by anaesthetizing them and blood was collected, serum was obtained by centrifuging the blood at 3000 rpm for 15 minutes and was kept at -80°C for further evaluation of serum biochemistry of thyroid or more (T3, T4 and TSH) gland. Thyroid gland was dissected out, cleaned, weighed and was fixed in Bouin's fixative for histopathological studies and the rest were kept for tissue biochemistry:

B. Assessment of thyroid gland weight index

The weight of thyroid gland was assessed to compare variations in weight between the experimental groups.

C. Thyroid gland tissue biochemistry (LPO, GSH and total protein content)

Thiobarbituric acid reactive substances (TBARS) are measured by the process of [28] with some modifications. The homogenate was prepared in Tris-HCl buffer and absorbance was measured at measured at 535nm. The thiol content of thyroid was measured following the method [29] with some modifications. The absorbance of supernatant was measured at 412 nm. Molar extinction coefficient of 13,100 was used to calculate thiol content.

Total protein content was quantified by spectrophotometric method [30] with some modifications and absorbance was taken at 625nm.

D. Thyroid Hormonal assays (T3, T4 and TSH). Assessment of thyroid hormones includes T3, T4 and TSH was estimated by using ELISA commercial kits following the instructions of the provided in the kits.

E. Histological preparation. Thyroid gland of all the experiment groups was fixed in Bouin's fixative for 24 hours. The tissues were cleared in different grades of alcohol and blocks were prepared in paraffin wax. Hematoxylin-eosin stained slides were observed under light microscope [31] for histopathological changes in thyroid tissues.

Stastical analysis. Results are expressed as mean ±S.E. Data was analyzed student's t-test followed by one-way ANOVA using IBM 20.0 version software [32]. Results were considered significant at different levels ($P \leq 0.05$, 0.01 and 0.001 implied significance).

IV. RESULTS

A. Assessment of weight index

Streptozotocin induced diabetic rats revealed significant decrement in weight of thyroid gland. However, melatonin treatment administrated to streptozotocin induced diabetic rats showed restoration in gland weight towards the control level. Melatonin alone does not cause any abnormal change in gland but regulated thyroid weight toward control (Fig. 1).

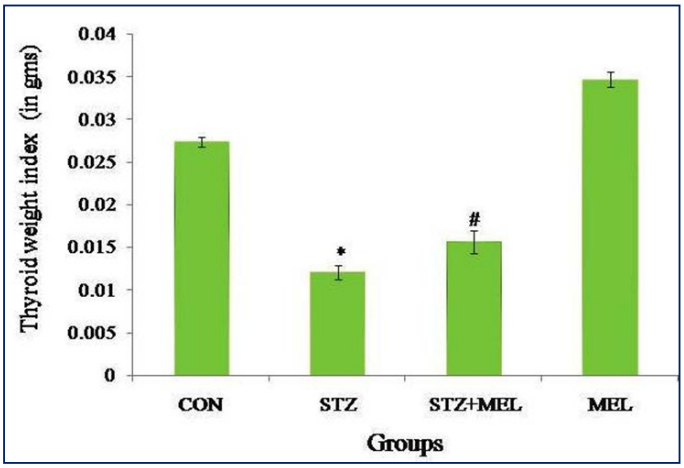

Fig. 1. Effect of melatonin (MEL) on weight of thyroid gland of Streptozotocin induced diabetic male rat showing significant decrease in organ weight in diabetic rats. Histogram represent Mean+SE; N=4. CON = Control, STZ= Streptozotocin, STZ+MEL= Streptozotocin +Melatonin, MEL= Melatonin.

B. Tissue biochemistry

The free radical production in thyroid tissues was noted higher in Streptozotocin induced diabetic rats. While as melatonin administration to Streptozotocin induced diabetic rats significantly decreased lipid peroxidation measured in terms of TBARS level. This decrease in lipid peroxidation might be due to free radical scavenging ability of melatonin. Melatonin alone does not showed any free radical generation in thyroid tissues. The non-enzymatic antioxidative system including reduced glutathione (GSH) revealed significant decrement in diabetic control animals, which indicated the diabetic condition down regulates the antioxidative system of the body of an organism. Administration of exogenous melatonin showed significant increment in GSH level by donating electrons by its indole ring to neutralize the free radicals and also provides H^+ to the oxidized glutathione (GSSG) to return in its reduced state. Free radical mediated oxidative damage to cellular membrane leads disintegration of lipid bilayer by stealing electrons from the polyunsaturated fatty acids and makes them unstable and ultimately they lose their property to maintain the selective permeability of the biomembranes. In STZ induced diabetic rats total cellular protein content was found significantly decreased while as animals given treatment of exogenous melatonin significantly restored the protein content near to the control level. Further, melatonin (*per se*) does not showed adverse change in total protein content (Table 1).

Table 1: Protective effect of exogenous melatonin against STZ induced alterations in metabolically important endocrine gland (thyroid gland) stress markers and total protein content.

Treatments	TBARS (nmol/mg of protein)	GSH (µg/mg of protein)	Protein µg/ml)
CONT	2.30±0.55	16.85±1.38	24.29±2.05
STZ	4.30±0.35$^{\$}$	7.56±1.43$^{\$}$	10.78±0.30$^{\$}$
STZ +MEL %Protection	3.06±0.37$^{\#}$ 62%	14.53±1.03$^{\#}$ 75.02%	16.29±1.33$^{\#}$ 40%
MEL	2.40±0.64	15.07±0.47	23.04±1.46
F Value	@3.501	@12. 884	@19.415

Data are mean ± S.E.; N=4; CONT=Control; STZ=Streptozotocin; MEL=Melatonin; TBARS; Thiobarbituric acid reactive species; GSH=Reduced Glutathione @ Significant at 5% for ANOVA. $^{\$}$STZ vs CONT.$^{\#}$STZ vs STZ + MEL at P≤0.05.

Serum biochemistry of triiodothyronine (T3), tertraiodothyronine (T4) and thyroid stimulating hormone (TSH)

In STZ induced diabetic rats showed significant reduction in T3, T4 and TSH level in circulatory serum. However, exogenous melatonin treatment given to the diabetic rats significantly increased the level of T3, T4 and TSH level near to the control level. Melatonin (*per se*) does not show any adverse change in T3, T4 and TSH level (Table 2).

Table 2: Protective effect of exogenous melatonin against STZ induced alterations in metabolically important endocrine gland (thyroid gland) T3, T4 and TSH level in serum.

Treatments	T3 (ng/dL)	T4 (ng/dL)	TSH (µIU/dL)
CONT	103.25±2.57	95.25±2.63	0.33±0.02
STZ	58.00 ±2.86 $^{\$}$	59±2.09 $^{\$}$	0.051±0.01 $^{\$}$
STZ +MEL %Protection	82. 75± 3.69$^{\#}$ 54.69%	78.75±4.27$^{\#}$ 54.48%	0.23±0.03$^{\#}$ 64.15%
MEL	100.75±1.94	94.25±3.64	0.29±0.05
F Value (at 5% level)	@ 54. 529	@ 27.020	@ 24.647

Data are mean ± S.E.; N=4; CONT=Control; STZ=Streptozotocin; MEL=Melatonin; T3; Triiodothyronine; T4 = Tertraiodothyronine; TSH = Thyroid Stimulating Hormone @ Significant at 5% for ANOVA. STZvs CONT.$^{\#}$STZ vs STZ + MEL at P≤0.05.

C. Histopathology

STZ induced diabetic rat revealed significant decrease in the number of follicles, while as melatonin administration significantly increased and restored follicular number towards the control level. Treatment of melatonin *per se* regulated the follicular number (Fig. 2).

V. DISCUSSION AND CONCLUSION

Diabetes mellitus is a multifaceted metabolic disorder characterized by incremental glucose level in blood (hyperglycemia) and secretion and synthesis of insulin becomes insufficient or insulin receptors does not respond to insulin produced by the pancreas inside the body [33]. It has been reported that during diabetes the islets of langherhens becomes shrinked, reduce in size, decrement in number [34].

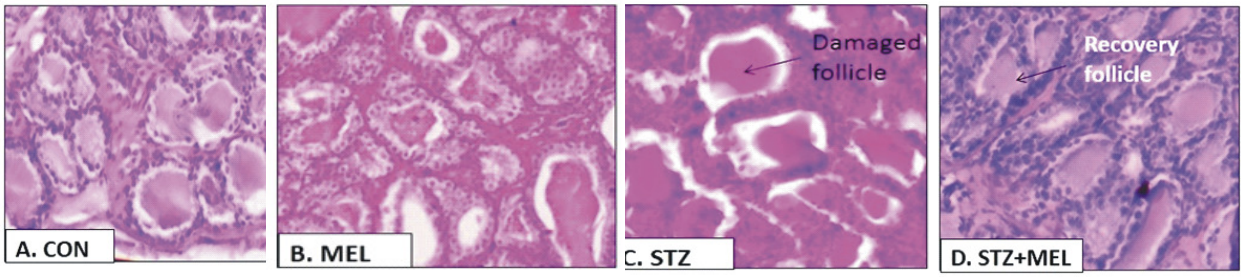

Fig. 2. Histomicrograph of thyroid tissues of STZ induced diabetic rat model showing effect of melatonin. A-ontrol showing normal number of thyroid follicles, B- Melatonin regulates the number of follicles, C- STZ treated group showing decreased number of follicles and D- STZ+MEL treated groups restored follicular number.

Thyroid gland has its key role in the metabolic homeostasis of the body [11]. Melatonin has been discovered to have its involvement in growth and secretory process of thyroid [35]. Thyroid gland weight index revealed significant decrease in STZ induced diabetic rats, while as treatment of exogenous melatonin given to the diabetic animals for four weeks significantly restored weight index near to the control level. The decrease in weight index was further supported by cellular disruptions and histological observations in which number of follicles showed significant decrease in diabetic animals. The result of the current study coincides with the previous findings of [36] who reported that diabetes causes follicular disruptions. The common diagnostic marker of diabetes, excess glucose gets autooxidized and is the cause of origin of free radical production. Lipid peroxidation is extensively used method to measure the oxidative stress in blood, serum or any other organ or tissue [37]. Oxidative stress is interrelated with numerous keys of adiposity and a low antioxidant defence [38]. Present study revealed significant increase oxidative stress in thyroid gland of diabetic rat. Previous studies reported that during diabetic conditions the lipid profile gets disturbed which makes more susceptible to peroxidative damaged by lipid peroxidation process. Diabetic control rats showed significant increase in lipid peroxidation level while as melatonin supplementation administered to diabetic rats showed significant decrement which explains the antioxidative (direct as well as indirect), free radical scavenging activity of melatonin and reactive nitrogen species (RNS) including H_2O_2, OH^-, O_2^- and NO^{-54}. It has been reported that reactive free radicals react with the amino acids and causes modification and denaturation of proteins [39]. Melatonin being a lipophilic molecule acts both ways receptor and non-receptor level neutralize the free radicals by donating electrons from its indolering, hence prevent the non-enzymatic protein glycosylation and protein denaturation. It is critical that cell maintains the level of reduced glutathione and low level of glutathione disulphide [40]. Glutathione reductase is an enzyme found in cell that converts glutathione disulfide back to reduced glutathione [32]. The oxidative stress lead breakdown of plasma membrane, due to intracellular content leak out from the cells into the extracellular environment. Total protein content was observed significantly reduced in STZ-induced diabetic conditions. Whereas as MEL treatment significantly increase protein content in streptozotocin-induced diabetic rats. The recovery in protein content might be because of MEL stimulates the insulin secretion and homeostasis which in agreement with [41], and its oral administration exhibits anti- hyperglycemic effect in young rats as insulin sensitizer and also improves functioning of β-cell [36] and also supported by clinical results indicate that MEL improves glycemic control in blood [41].

The insulin and thyroid hormones dependent on each other, as diabetes mellitus and thyroid diseases are two common endocrine mostly in adults [42]. Uncontrolled diabetes (typ-I and type-II) is characterized by low T3 level, TSH concentration [43] and TSH losses it affinity/response to TRH. Decreased level of T3 is due to decreased conversion of thyroxine (T4) to triiodothyronine (T3) in periphery via 5' monodeiodination reaction. In present study, significant decrease in T3, T4 and TSH level in blood serum. This might be due to reduced conversion of T4 to T3 and increased formation of rT3 because activity of 5'-deiodinase decreases due to reduced action of 5´-deiodinase [42]. Melatonin seems to enhance the action of 5´-deiodinase and increase the formation of T3. Diabetic control rats revealed significant decrement in thyroxin supported by histological observations in which number of follicles showed significant reduction which coincides with previous findings of [44]. TSH level revealed significant decrease which in concurrent reduction in free thyroxine level in serum supported by earlier studies of Shah, (2007) [13]. The affinity of TSH towards the TRH is one of the main factor responsible for reduction in TSH during diabetic condition. The findings of the present study reports that the melatonin might have role in regulation of TSH level along with the Deiodinase activity. The decline in oxidative stress by melatonin treatment might be a novel therapeutic approach against the STZ induced diabetic thyroid gland oxidative stress and hormonal imbalance. The reduced TSH level in the diabetic condition might be caused by the oxidative damage in the thyrotrophs. Furthermore, that also might be caused by the oxidative stress in the hypothalamic region. Our study is in agreements with earlier studies which reported that diabetes induced by chemical agents in animals alters the hypothalamo-pituitary–thyroid axis which causes decrement in hypothalamic and plasma thyrotrophin-releasing hormone (TRH). Pituitary and plasma thyroid-stimulating hormone (TSH) and also declined tri-iodothyronine (T3) and thyroxine (T4) synthesis [45,14]

Histopathological studies revealed reduction in thyroid follicles in STZ induced diabetic rats, however, melatonin administration significantly restored the follicular population towards the control level.

Melatonin (*per se*) does not showed any adverse change in follicular number and morphology of the thyroid gland the histological studies were supported by previous findings of Histological observations of thyroid gland revealed that in case of diabetic rat the number of follicles gets reduced and exogenous melatonin administration restores the cellular architecture of thyroid gland comparable to the normal architecture. From the results and observations of the present findings it might be concluded that melatonin based diabetic therapy could control the diabetic complications in the metabolically involved thyroid gland, as thyroid gland is engaged in regulation of body growth, temperature regulation and calcium metabolism in the body of every organism.

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CONFLICT OF INTEREST

The authors confirm that there are no known conflicts of interest associated with this publication.

CONTRIBUTIONS

Dr. SR and YAH was involved in the conception and design of the study. YAH and MB was involved in data analysis and interpretation, dosing and drafting. HG participated in the drafting, data analysis of the manuscript. ND was involved in the biochemical evaluation and preparation of histology slides. All authors read and approved the final manuscript.

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