Original Article

Six Week Melatonin Therapy Improves the Triglyceride to HDLc Ratio and Prevents Atherogenic Tendency

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ABSTRACT

Objective: To observe the effects of six weeks melatonin oral therapy on TAGs/HDLc ratio and atherogenic tendency in alloxan induced diabetic rat model.

Study Design: Experimental study

Place and Duration of Study: This study was conducted at the Department of Anatomy, Pharmacology, and Pathology, SRMC, Tando Adam from May 2019 to December 2020.

Materials and Methods: A sample of one hundred male Wistar albino rats was divided equally into five groups. Group A (negative control) and B (positive diabetic control) were untreated. While Experimental groups D, E and F were treated with melatonin 5, 10 and 15 mg/Kg bwt daily orally for six weeks. Alloxan (120 mg/Kg body weight) was injected (i.p) to induce diabetes mellitus. Blood samples were analyzed for biochemical testing. Log TAG/HDLc ratio was defined as atherogenic index of plasma (AIP). Data variables were analyzed statistically (SPPSS ver. 21.0) at 95% CI ($p \leq 0.05$).

Results: Six weeks melatonin therapy significantly ameliorated the glycemic control (glucose, A1C, fasting insulin and C – peptide), blood lipids (Cholesterol, TAGs, LDLc and HDLc) of experimental diabetic rats (P=0.0001). TAGs/HDLc ratio (atherogenic index of plasma (AIP) was significantly improved after six weeks melatonin therapy (P=0.0001).

Conclusion: We report six week melatonin therapy improves triglyceride to HDLc ratio thus preventing the atherogenic tendency.

Key Words: Melatonin, Lipid profile, TAG/HDLc ratio, Atherogenicity

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INTRODUCTION

Melatonin is an amine hormone of pineal gland released at night time. Melatonin produces multiple biological effects.[1,2] Melatonin functions through its receptors on target cells. Melatonin receptors (MT) are two types - MT1 receptor is linked with brain functions while the MT2 receptor modulates the body's circadian rhythms.[2] Melatonin exerts neuroprotective and cardioprotective effects.

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Melatonin plays role in female and male reproductive system, synchronizing circadian rhythms, sleep cycle, metabolism, ocular functions, etc. Melatonin interacts with cell through its membrane receptors and intracellular mechanisms altering broad spectrum of physiological functioning vital for organism survival including cell metabolism, apoptosis, biological rhythms, etc.[3-5] Melatonin is also produced in other tissues such as the gonads, gut, bone marrow, retina, skin, astrocytes and lymphocytes, etc.[6] Synthesis of melatonin is reportedly controlled by postganglionic sympathetic nerves. Thus the melatonin is ubiquitously acting hormone.[7] Immunomodulatory, anti – inflammatory and anti – oxidative effects of melatonin are noticeable. Most vital physiological function is in the sleep wake cycle. It regulates the biological clock, immune response, and control of seasonal reproduction in lower animals.[3,8] Melatonin is reported regulate the body weight and energy balance.[3] Several metabolites of melatonin are effective scavengers thus making it an effective anti – inflammatory and anti – oxidant agent. Various food stuffs contain melatonin such as the herbs, olive oil, fruits, and vegetables. FDA classified melatonin as food supplement free from any serious adverse effects and toxicity.[1,3] Currently, the DM is

increasing in the country[9] with associated risk of metabolic derangements such as the dyslipidemia and atherogenicity. Altered blood lipids increase the tendency of vascular events due to atherosclerosis including the coronary vascular disease, peripheral arterial disease, coronary artery disease and cerebrovascular injuries, etc.[3,9] The present experimental study investigated the effects of melatonin therapy on TAGs/HDLc ratio and atherogenic tendency (AIP) in Alloxan induced diabetes mellitus in male Wistar albino rat model.

MATERIALS AND METHODS

The present experimental study, analyzing the therapeutic efficacy of melatonin, was approved by the Ethical Review Committee (ERC) of Suleman Roshan Medical College. Experiment covered duration from May 2019 to December 2020 and conducted at the Department of Anatomy, Pharmacology, and Pathology, SRMC, Tando Adam from May 2019 to December 2020. We purchased one hundred adult male albino rats (Wistar strain) form animal house according to inclusion and exclusion criteria. Inclusion criteria were declared as; male rat, body weight 150 – 200 gram, Albino Wistar strain. Animal feeding well and moving around the cages, looking healthy and active were as per inclusion criteria. While lazy male rats, sick, not feeding well, not moving actively and female sex were decided for exclusion from study protocol. Equipped animal house was ensured for all necessary requirement and animals housing was in according to the NIH Guidelines (Animal Research). 12/12 hour dark/light cycle was maintained strictly. Rats were examined on daily basis. Proper ventilation was ensured. Rats were given chow feeding twice daily. Control group A (n=20) were tagged as negative control – as DM was not inducted and was given 9.0% N/Saline as placebo therapy. Group B (n=20) was tagged as positive control – DM was inducted with Alloxan but therapy was not given. This group was used for comparison of research variable results of experimental diabetic melatonin treated groups (Groups C, D and E). Alloxan was purchased from Sigma Aldrich (USA). Alloxan dose of 120 mg/Kg[9] body weight was administered into the peritoneal cavity in fasting rats for DM induction. A rat attained random blood glucose level ≥250 mg/dl[9] was termed as diabetic rat. Rats showing successful DM induction were divided into Positive control B and Experimental groups D, E and F. Diabetic experimental groups D, E and F received melatonin 5, 10 and 15 mg/Kg bwt therapy. Melatonin was administered orally mixed diet daily orally for six weeks. After six weeks melatonin therapy, the rats were kept on fasting for six hours. Rats were anesthetized with ethylene ether in plastic containers. Comatose rats were pricked with capillary tube in the retro-orbital space to collect blood samples. Blood was centrifuged (x3000 rpm for fifteen minutes). Squeezed sera were preserved at – 20 ^{0}C. Sera were used for the biochemical analysis of study research variable of glycemic control – glucose, glycated hemoglobin (A1C), fasting insulin and C – peptide using standard clinical laboratory methods. Lipids profile was estimated on Cobas chemistry analyzer. TAG/HDLc ratio (AIP) was calculated as; Log TAG/HDLc ratio (AIP) determined by manually using scientific calculator. Scaling of TAG/HDLc ratio (AIP) used was; low risk 0.3 – 0.1, medium risk 0.1 – 0.24 and high risk >0.24 of atherogenic tendency.[10] Biochemical parameters of research interest were analyzed on SPSS ver. 21.0 using appropriate statistical tests (one – way analysis of variance and LSD Fischer`s post- Hoc test). Level of significance among group differences was taken at 95% CI ($p \leq 0.05$). Graphs were plotted on Microsoft Excel sheet.

RESULTS

Six weeks melatonin therapy significantly ameliorated the glycemic control (glucose, A1C, fasting insulin and C – peptide), blood lipids (Cholesterol, TAGs, LDLc and HDLc) of experimental diabetic rats (P=0.0001) (Table –1) (Graph – 1). TAGs/HDLc ratio (atherogenic index of plasma (AIP) was significantly improved after six weeks melatonin therapy in experimental diabetic rats (P=0.0001) (Graph – 2).

Table No.1: Biochemical findings and TAG/HDLc ratio (n=100)

	Group-A	Group-B	Group-C	Group-D	Group-E	P
Body weight (g)	158.3±25.3	131.3±34.1	135.3±13.1	141.3±12.3	151.4±11.5	0.95
Glucose (R) (mg/dl)	114.6± 6.9	352.4±40.3	285.1± 48.2	261.6±49.0	207.6±29.4	0.003
A1C (%)	4.08±1.1	8.6±0.7	7.3±1.5	6.65±1.1	6.0±0.58	0.016
Insulin (F) (µU/L)	6.2±0.5	2.6±1.2	4.5±1.1	4.7±1.0	5.3±0.4	0.018
C- peptide (mg/dl)	2.1±0.7	0.5±0.3	1.2±0.36	1.32±0.2	1.58±0.3	0.018
Cholesterol (mg/dl)	108.1±5.2	223.0±58.2	196.9±35.8	176.7±54.2	121.6±15.5	0.018
TAGs (mg/d)	109.4±6.0	176.6±33.0	191.0±31.7	156.8±25.3	139.4±22.3	0.015
LDLc (mg/d)	91.2±11.0	139.3±36.0	123.1±20.1	123.0±21.0	111.8±5.7	0.001
HDLc (mg/d)	39.0±3.6	21.9±3.3	25.0±2.3	30.5±3.0	32.7±4.3	0.001
TAG/HDLc ratio (AIP)	0.03±0.01	1.11±0.1	0.74±0.21	0.59±0.23	0.39±0.10	0.0001

AIP- Atherogenic Index of Plasma

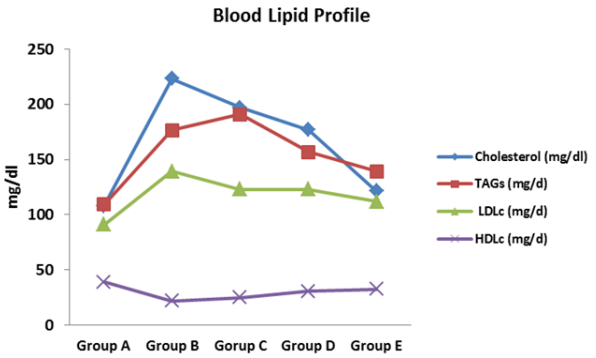

Figure No.1: Graph showing the blood lipid profile levels

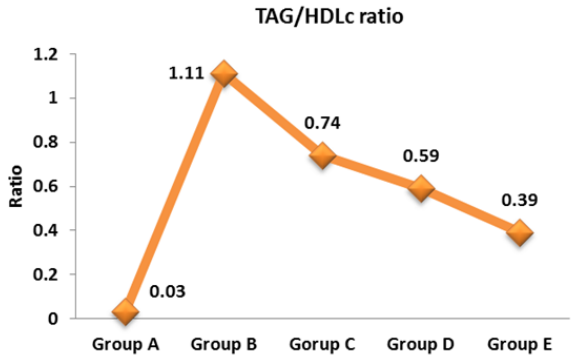

Figure No.2: Graph showing the TAG/HDLc ratio (AIP)

DISCUSSION

Melatonin is the chief hormone of the pineal gland that interacts with a variety of different cells.[1] In humans, melatonin receptors has been detected in the retina, brain, suprachiasmatic nucleus, central and peripheral arteries, kidneys, pancreas, adipocytes and immune cells.[12,13] It is known that melatonin exerts antioxidant, anti-inflammatory, anti-hyperlipidemic and antihy-pertensive actions and also modulates insulin secretion and action.[14,15] There are reports showing that melatonin–insulin interactions and relationship between melatonin–insulin ratio and lipid profile may exist in patients with metabolic syndrome[16] and that melatonin therapy improves blood pressure, lipid profile and parameters of oxidative stress in patients with metabolic syndrome.[17] The present study has demonstrated that melatonin treatment of obese rats was associated with a reduction in body weight without affecting food intake. We observed six weeks melatonin therapy significantly improved the glycemic control (glucose, A1C, fasting insulin and C – peptide), blood lipids (Cholesterol, TAGs, LDLc and HDLc) and TAGs/HDLc ratio (atherogenic index of plasma (AIP) in the experimental diabetic rats (P=0.0001). Log TAG/HDLc was taken as atherogenic tendency.[9] The findings of present study are in agreement with previous reports.[1-3] A previous study[18] observed the melatonin decreased blood cholesterol levels in genetically mutated hypercholesterolemia rats with improvement of fatty liver. Another previous study[19] analyzed the fructose fed rats with melatonin therapy and found dyslipidemia was attenuated in experimental rats. A previous animal study[20] conducted on rats, observed the melatonin therapy attenuated the lipid peroxidation. Serum Cholesterol, TAGs, FFA and phospholipids were decreased to normal in the brain and liver rat. A previous study[21] conducted on normal rats found melatonin intake improved total plasma lipids and hepatic phospholipids and increase in n-6 polyunsaturated fatty acids (PUFA) was observed. A recent review by Danilenko et al[1] (2019) has critically proved lipid lowering potential of melatonin. A previous study[22] administered 10 mg melatonin to 14 patient's diagnosed idiopathic hypercholesterolemia. They found improvement of blood lipid concentration with significant rise in serum HDLc similar to present study (table 1). However, findings of previous studies[23,24] conducted in post menopause women with two weeks melatonin therapy (6 mg) found deterioration of TAGs and VLDL levels. The findings are inconsistent to present and previous studies.[20-22] Another clinical study[24] analyzed 14 elderly women with melatonin therapy for six months, and reported no change in the blood lipids compared to baseline. Inconsistent findigns of above studies[23-25] are most probably due to the small sample of study subjects with altered milieu of hormone in post menopause hence findigns are incomparable. A previous study[26] investigated the effects of melatonin therapy in obese rats and found TAGs and LDLc were decreased, HDLc was increased but observed no changed in total cholesterol. The forming finings of TAGs, LDLc and HDLc are concordant to our present study; however insignificant cholesterol change is discordant to present and other previous studies.[22-22] Evidence based findgins of melatonin therapy in induced diabetic rats of present experimental study are highly significant that may be exploited for treating the hyperlipidemia and dyslipidemia of diabetes mellitus patients and atherogenic tendency may be prevented, however this needs further experimental and clinical trials in indigenous populations to validate the findings for making melatonin available using in clinical practice.

CONCLUSION

We report six week melatonin therapy improves triglyceride to HDLc ratio thus preventing the atherogenic tendency Melatonin improves the blood lipids, glycemic control and atherogenic index of plasma (AIP) in alloxan induced diabetes in male Wistar albino rat model. Further experimental studies and clinical trials are warranted for in – depth studies to reach to proper benefits of melatonin therapy making

this natural remedy available for clinical use for preventing the atherosclerosis.

Author's Contribution:

Concept & Design of Study:	Aftab Abbasi
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Revisiting Critically:	Aftab Abbasi, Hina Mawani
Final Approval of version:	Aftab Abbasi

Conflict of Interest: The study has no conflict of interest to declare by any author.

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