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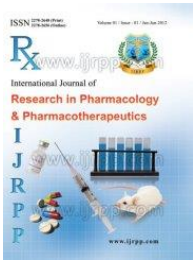

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Effect of melatonin on body weight, plasma lipid, leptin and insulin levels in high fat diet fed rats

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ABSTRACT

Aims and Objectives

To understand the influence of melatonin on body weight, plasma lipid, leptin and plasma insulin levels.

Materials and Methods

A total of 36 laboratory bred male Sprague dawley rats were used for the experiment. All the baseline parameters like body weight, lipid levels and plasma leptin and insulin levels were measured. The rats were fed with high fat diet starting from day 0 till 6 weeks. These rats were divided into two groups of 18 each. Group 1 (Control) received normal saline and Group 2 (Test) received 0.4mcg/ml of melatonin via oral gavage tube daily for six weeks. After six weeks, the parameters like Total body weight, lipid profile, plasma leptin and insulin levels were again observed. The statistical analysis of data was done using students't' test, one way or two way ANOVA followed by post hoc bonferroni multiple comparison tests using SPSS. P value less than 0.05 was considered to be significant.

Results

The average weight gain in control group fed with fat rich diet was increased by 6% but the melatonin supplementation suppressed this increase to only 1.03%. Further, melatonin supplementation also suppressed the rise in total cholesterol, LDL, triglycerides and HDL cholesterol significantly ($P<0.05$). Melatonin administration not only suppressed the rise but also decreased the plasma leptin and insulin levels by 32% & 18.9% respectively after six weeks.

Conclusion

Daily supplementation of melatonin for six weeks in Sprague dawley rats fed with high fat diet suppresses the rise in body weight, plasma lipid levels and also decreases leptin and insulin levels significantly.

Keywords: SD Rats, Cholesterol, Lipids, Fats, Circadian rhythm, Metabolic syndrome

INTRODUCTION

Melatonin is an amine of molecular weight 232 that is synthesized from tryptophan, which is an essential amino acid, produced via intermediate serotonin. It has been regarded as a specific hormone of the pineal gland, but is actually produced in various locations like retina, brain (cerebral cortex, raphe nuclei, striate body, etc.), gastrointestinal tract (stomach, small intestine, etc.), testes, ovaries, spinal cord, lens and skin. Melatonin, not only in vertebrate and invertebrate animals, but is widely distributed in plants such as rice, barley, and wheat. [1] Melatonin is secreted from the pineal gland at night. It has various physiological, immunological, and biochemical functions. Melatonin acts as circadian mediator. Melatonin is also able to eliminate free radicals such as reactive oxygen species. Along with these functions, Melatonin also has sleep promoting effect. Melatonin administration lowers deep body temperatures in those with rhythm disorders and also in healthy individuals of all age groups, from children to elderly people. Melatonin shortens the time required to fall asleep; and improves sleep. [2]

In the past 15-20 years numerous studies have been undertaken for the potential uses of melatonin in medicine. The only established use of melatonin or its agonists as of now is in sleep disorders like jet lag but has shown promising results in variety of related disorders like inability to fall asleep (insomnia); delayed sleep phase syndrome (DSPS); rapid eye movement sleep behavior disorder (RBD); insomnia associated with attention deficit-hyperactivity disorder (ADHD); insomnia due to certain high blood pressure medications called beta-blockers; and sleep problems in children with developmental disorders including autism, cerebral palsy, and intellectual disabilities. It is also used as a sleep aid after discontinuing the use of benzodiazepine drugs and to reduce the side effects of stopping smoking, a chronobiological disorder. [3]

The lesser focused area is the effect of melatonin in metabolic disorder. As of now there are few studies showing the effects of melatonin on body weight, adiposity, dyslipidemia, oxidative stress, leptin and insulin levels. Melatonin reduces body weight gain in rats with diet induced obesity [3] and increases energy expenditure. [4] It also improves lipid profile by decreasing triglycerides, LDL and increasing HDL without affecting total cholesterol levels. [5] It improves antioxidant status. [6] Melatonin decreases plasma leptin levels as well as plasma insulin levels. [7]

Melatonin is a recent discovery. It has yet to go through the extensive studies which most hormones and other dietary supplements go through before it can be approved for clinical use. It seems to have many positive effects on the body. This experiment is an attempt to study and establish the effects of melatonin on body weight, lipid profile and insulin levels which are the major component of metabolic syndrome / syndrome X.

MATERIAL AND METHODS

Animals

Laboratory bred male Sprague dawley rats, weighing between 440-480 g were procured from palamoor biosciences, mahabubnagar. All the animals were individually housed in standard 10.5 × 19-in. clear plastic cages and were maintained under standard laboratory conditions at 25°C with water ad libitum and normal photo period (12hr dark/12hr light). Experimental protocol has been approved by the Institutional Animal Ethics Committee (IAEC).

Drugs and Chemicals

Melatonin powder (of >98% purity) manufactured by sigma aldrich lab with batch number M5250 was used for the study.

Experimental design

A total of 36 rats were used for the experiment. All the baseline parameters like body weight, lipid levels and plasma leptin and insulin levels were measured. All these animals were fed with high fat diet [fat content 42% of energy, based on lard (HF-L), olive oil (HF-O), coconut fat (HF-C) or fish oil (derived from cod liver, HF-F).] starting from day 0 till 6 weeks. These animals were divided into two groups of 18 each. Selection and grouping was based on randomization using the excel programme. Group 1 (Control) received normal saline and Group 2 (Test) received 0.4mcg/ml of melatonin via oral gavage tube daily for six weeks. After six weeks, on the day of sample collection, the distal tip (<1 mm) of the tail was snipped with sharp surgical scissors, and blood was gently milked into a heparinized capillary tube; plasma was then separated by centrifugation and stored at −70 C. The parameters like Total body weight, lipid profile, plasma leptin and insulin levels were again observed.

Statistical Analysis

The statistical analysis of data was done using students't' test, one way or two way ANOVA followed by post hoc Bonferroni multiple comparison tests using SPSS. P value less than 0.05 was considered to be significant.

OBSERVATIONS AND RESULTS

All the rats were weighed before and every week for six weeks of continuous melatonin administration. The results are shown in table 1.

Table 1: Weekly changes in the total body weight of rats after continuous melatonin administration

Groups	Total body weight (in grams)						
	0 weeks	1 week	2 week	3 week	4 week	5 week	6 week
Control	453.84±2.73	459.24±3.78	465.59±4.55	473.15±4.65	475.95±4.86	479.07±5.08	481.55±5.32
Test	454.62±3.72	456.02±2.21	456.25±2.60	457.12±2.25	458.32±2.38	458.01±2.15*	459.32±2.32*

All values are expressed as Mean±SEM, n=18, *P<0.05

The lipid profile was done for every rat before and after six weeks of continuous melatonin administration. The results are shown in table 2 and figure 1.

Table 2: Changes in the lipid profile in rats after six weeks of continuous melatonin administration

	Total cholesterol (in mg/dl)		Triglycerides (in mg/dl)		HDL (in mg/dl)		LDL (in mg/dl)	
	0 weeks	6 week	0 weeks	6 week	0 weeks	6 week	0 weeks	6 week
Control	65.48±2.32	72.93±3.45	75.67±1.25	92.00±2.84	22.32±2.25	23.18±1.75	38.95±3.25	46.25±2.14
Test	63.54±2.70	69.27±3.15	77.35±2.14*	80.28±3.14	21.28±2.15	26.25±1.98	38.24±2.96	39.35±2.45*

All values are expressed as Mean±SEM, n=18, *P<0.05

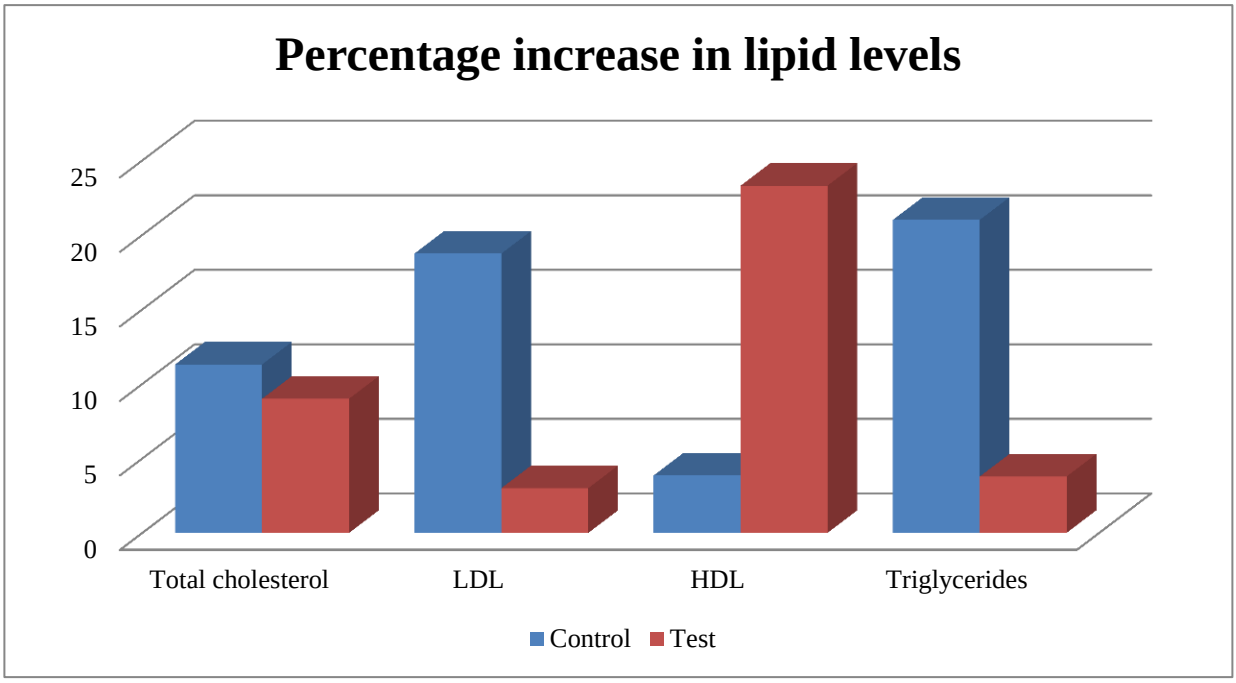

Figure 1: percentage increase in lipid levels after six weeks of fat rich diet with or without melatonin in rats

The plasma leptin and insulin levels in each rat were measured before starting the experiment and after six weeks of continuous melatonin administration. The results are shown in table 3.

Table 3: Effect of six weeks of continuous melatonin administration on plasma leptin and insulin levels in rats

	Plasma leptin levels (in µg/ml)		Plasma insulin levels (in ng/ml)	
	Before	**After**	**Before**	**After**
Control	6.15±0.85	7.34±1.20	1.34±0.15	1.49±0.84
Test	6.23±0.65	4.24±1.15 *	1.38±0.21	1.12±0.65 *

All values are expressed as Mean±SEM, n=18, *P<0.05

DISCUSSION

In the present, the effect of daily melatonin administration on total body weight, plasma lipid levels, plasma leptin and insulin levels were studies. The average total body weight of rats before treatment in control and test group was almost similar. After feeding rats in control group with high fats diet for six weeks, the weight started increasing as it was obvious in weekly weight measurements. The maximum average weight gain in control group after six weeks was about 6%. But, when the same high fat diet was given to the rats in test group along with daily melatonin administration the average weight gain was just 1.03%. This indicates that melatonin significantly (P<0.05) suppressed the total body weight gain in high fat fed SD rats compared to control rats. The results are in consistent with previous studies by Puchalski SS et al [8] which also showed similar effect. They observed that, melatonin and control rats increased body weights while consuming the high-fat diet; melatonin rats gained approx 4% during the first 2 week and then stabilized, whereas control rats continued to gain for an additional week, achieving 8%gain before stabilization. After the full 8 week of treatment, body weights of the melatonin rats remained significantly lower than those of control rats. Another study by T. Wolden-Hanson et al [9] also showed that daily Melatonin administration to middle aged male rats suppresses body weight independent of food intake and total body fat. A study done by BénédictePrunet-Marcassus et al [10] also showed that melatonin reduces body weight gain in spraguedawley rats with diet-induced obesity. Here, they had given melatonin only for 3 weeks.

We also studied the effect of melatonin on lipid profile. In the control group, the average total cholesterol was 65.48±2.32 mg/dl, LDL was 38.95±3.25 mg/dl and triglycerides were 75.67±1.25 mg/dl. After six weeks of high fat diet, the average total cholesterol was increased to 72.93±3.45 mg/dl, LDL increased to 46.25±2.14 mg/dl and triglycerides increased to 92±2.84 mg/dl. But, when melatonin was given to the rats in test group, it suppressed the rise in total cholesterol, LDL and triglycerides significantly. Here, the rise in LDL was only about 3% (compared to 18.74% in control group), rise in triglycerides was only about 3.78% (compared to 21% in control group) but, the total cholesterol continued to rise about 9% (compared to 11.37% in control group) after six weeks. The melatonin however didn't show any significant suppression in rise of total cholesterol whereas, it showed significant suppression (P<0.05) of LDL and triglycerides. Melatonin also significantly (P<0.05) increased the average HDL cholesterol levels. There are many studies [10, 11 and 12] showing the reduction in lipid levels (Total cholesterol, LDL and triglycerides) by melatonin administration in already obese/dyslipidemic/diabetic rats, but there are few researches which have studied the suppression of rise in lipid levels by high fat fed diet in rats. One such study by Maria J Rios-Lugo et al [13] showed that melatonin attenuated the increase in triglycerides and cholesterol levels.

The next parameters studied were leptin and insulin. The foregoing result suggests that the concomitant administration of melatonin for six weeks to the high fat fed rats not only suppressed the rise but also decreased the plasma leptin and insulin levels by 32% & 18.9% respectively. But in the control group the high fat fed rats showed increase (19.35% & 11.21% respectively) in leptin and insulin levels which were not significant compared to baseline reading. Providing high fat feed to the rats induces obesity like state and increases leptin and causes insulin resistance [14]. Daily melatonin

administration in these rats suppresses leptin and insulin rise and restores to their youthful levels [8, 11 and 15].

The exact mechanism for suppressing the rise in body weight by melatonin in the absence of significant differences in food intake in the rats needs to be further explored. A key piece of evidence in this respect is the observation that melatonin plays a fundamental role in the seasonal changes of adiposity of Siberian hamsters by increasing the activity of the sympathetic nervous system innervating white fat, thereby increasing lipolysis [16]. May be similar mechanism exists in rats. The exact mechanism how melatonin decreases cholesterol levels is also not clear. If established in future research, these findings suggest that the pineal gland may exert a cholesterol reducing effect and that melatonin could be used therapeutically in the treatment of hypercholesterolemia. In one of the study by Kus L et al [15], it is proved by immunohistochemical studies that Pinealectomy increases and exogenous melatonin decreases leptin production in rat anterior pituitary cells. The main source of leptin is adipose cells, both leptin and insulin levels tend to parallel adipose tissue mass, [16 and 17] melatonin by decreasing adiposity directly reduces leptin level.

In summary, we have shown that supplementation of melatonin in rats fed with high fat diet suppresses the rise in body weight, decreases plasma lipid levels, leptin and insulin levels. Several other studies have also shown that melatonin decreases intra-abdominal adiposity, adiponectin levels, acts as anti-oxidant preventing lipid peroxidation [18 and 19]. In metabolic syndrome we see, obesity, dyslipidemia, insulin resistance and hypertension. So, in metabolic syndrome, there might be decrease in endogenous melatonin secretion which alters energy regulation, resulting in increased body weight and adiposity and their associated detrimental metabolic consequences. However, it is important to note that extrapolating from these findings to species other than the rats is not currently warranted. Further, melatonin's role in cardiovascular risk factors such as obesity and other related disorders including the metabolic syndrome needs further investigations, particularly in humans before they can be advised in such diseases.

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