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Physiological Effects Of Melatonin on Leptin, Testosterone and Biochemical Parameters in Albino Rats

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***Abstract**: The Objective For The Present Study Is To Investigate Of The Effects Of Melatonin (Mel) On Serum Leptin, Testosterone, Liver Function Test Parameters In Control And Pinealectomized (Pinx) Rats. Thirty Seven Adult Male Rats Were Used In This Study. The Experimental Rats Were Divided Into Six Groups, And The Treatments Were Continued For Six Weeks As The Following: Group 1: Control Rats. Group 2: Sham-Operated Surgery Rats. Group 3: (Pinx) Rats. Group 4: Pinx Rats + Melatonin (60 Mg / Kg Diet). Group 5: Melatonin (60 Mg / Kg Diet). Group 6: Melatonin (120 Mg / Kg Diet). Reduction In Mel Secretion Was Induced By Removing Of Pineal Gland (Pinealectomy). Results Showed That Pinealectomy And Mel Treatment At High Dose In Control Rats Significantly Reduced Serum Leptin Concentration, But Mel At Low Dose Administration In Pinx And Control Rats Significantly Elevated Serum Leptin Concentration. On The Other Hand, Both Doses Of Mel Significantly Decreased Serum Testosterone Concentration. Serum Alanine Aminotransferase (Alt) And Aspartate Aminotransferase (Ast) Activity And Malenaldehyde (Mda) Levels Increased In Pinx Rats. On The Other Hand, Mel (60 Mg / Kg Diet) Administration Decreased Serum Transaminases Activity And Mda Level Toward Normal Activity In Pinx Rats. In Conclusion: Melatonin Has Important Roles In The Control Of Leptin Production And The Regulation Of Reproductive System (By Decreasing Testosterone Concentration) ,Also ,It Decreased Serum Transaminases In Pinx Rats And This Suggests That Melatonin Has A Hepatoprotective Role*

***KEYWORDS:** melatonin, leptin, testosterone, oxidative stress.*

I. INTRODUCTION

Leptin is a hormone, discovered in 1994 by Jeffrey M. Friedman and colleagues at the Rockefeller University. It is a secreted protein that is encoded by the *ob* gene, the *ob* gene is highly conserved among vertebrate deoxyribonucleic acid (DNA) (Zhang *et al*., 1994).

Leptin, originally identified in adipocytes, it plays an important role in the regulation of food intake and energy balance (Archanco *et al*., 2003). Results of several studies have suggested that leptin may also play a role in the regulation of metabolism, sexual development, reproduction, heamatopoiesis, immunity, gastrointestinal functions, sympathetic activation and angiogenesis (Baltaci and Mogulkoc, 2007).

Functional pinealectomy (continuous light exposure) represents a more physiological model of reduced MEL levels as compared to surgical pinealectomy (removal of epiphysis), which decreases not only the night-time MEL level but also the day-time MEL level (Vazan *et al*., 2007).

Melatonin a biologically active molecule, is one of the neurohormones (Darul and Kruczynska, 2004). It is secreted rhythmically and affected by environmental photoperiod (Donmez *et al*., 2004).

In rodents, the putative role of MEL in the modulation of glucose metabolism has been investigated using surgical pinealectomy to cause the chronic inhibition of endogenous MEL synthesis, or by administering exogenous MEL (Kosa *et al*., 2001). Some studies have reported that MEL treatment increases glycaemia in rats, whereas ,other studies showed no effect, or even a decrease (Iizuka, 1996).

In addition, MEL exerts protective and therapeutic effects against liver injury through its antioxidant action (Kiarostami *et al*., 2006). Wang *et al*., (2004) reported that MEL has a protective effect against liver injury in mice and decreased significantly serum AST and ALT, they related this decrease to its free radical scavenging, increased superoxid dismutase (SOD) activity and pro-inflammatory mediators.

Beside these functions, it has been suggested that MEL may have an effect on leptin release (Bojkova *et al*.,2006). It has been shown that MEL and leptin are secreted with circadian rhythms so that their levels are low during the day and high during the night (Mastronardi *et al*., 2000).

Some studies suggest that both exogenous and endogenous MEL inhibit leptin levels or that pinealectomy increases leptin levels (Canpolat *et al*., 2001), whereas, others argue that pinealectomy suppresses leptin levels (Alonso-Vale *et al*., 2004). Also, it has been suggested that MEL can produce different effects on leptin levels depending on the period of administration, age and/or dose (Rasmussen *et al*., 2001).

Sirotkin and Shaeffer, (1997) suggested that MEL exerts its antigonadal effects, at least in part, through the direct decrease of testosterone production. Also, Kus *et al*., (2002) indicated that pinealectomy induced increased testosterone secretion in Leydig cells and this increased secretion can be prevented by administration of MEL. It has been found that MEL beside its function as a broad spectrum free radical scavenger, MEL may also limits the MDA levels (Dobsak et al., 2003). The objectives for the present study is to investigate the effects of MEL on serum leptin, testosterone, liver function test parameters in control and PINx rats.

Materials and Methods

Animals and housing

Thirty seven adult male albino rats were used in this study. All rats were weighing about (300-350gm) and (9-11) weeks of age at the time when the experiment started. Animals were housed in plastic cages bedded with wooden chips. They were housed under standard laboratory conditions, 12:12 light/dark photoperiod at 22 ± 2 ºC (Coskun *et al*., 2004). The animals were fed on standard rat chow and tap water *ad libitum.*

Experimental Design

This experiment was designed to study the effect of two doses of melatonin on serum leptin, testosterone and some biochemical parameters (serum ALT, AST, total protein, albumin, uric acid, MDA and blood glucose) in male albino rats. Animals were assigned randomly to six different treatments and were continued for 6 weeks as the following:

Group 1: Control rats. The rats were given a standard rat chow and tap water *ad libitum*.

Group 2: Sham-operated surgery rats. The rats underwent sham-operated surgery and given standard rat chow and tap water *ad libitum*. .

Group 3: PINx rats. The rats of this group underwent pinealectomy and given standard rat chow and tap water *ad libitum*.

Group 4: PINx rats + melatonin (60 mg / Kg diet). The rats of this group underwent pinealectomy and were given standard rat chow supplemented with melatonin (60 mg / kg diet).

Group 5: Melatonin (60 mg / Kg diet). The rats were given standard rat chow supplemented with melatonin (60 mg / kg diet).

Group 6: Melatonin (120 mg / Kg diet). The rats were given standard rat chow supplemented with melatonin (120 mg / kg diet).

Collection of blood samples

At the end of each experiment, the rats were anesthetized with ketamine hydrochloride (50 mg/kg). Blood samples were taken by cardiac puncture into chilled tubes with ethylene diamine tetra acetic acid (EDTA) (4.5mM) as anticoagulant and centrifuged at 3000 rpm at 0°C for 15 minutes; then sera were stored at -80°C (Sony, Ultra low, Japan).

Biochemical determination

Determination of serum leptin and testosterone concentration

Serum leptin and testosterone concentrations were determined by using enzyme linked immunosorbent assay (ELISA) kit.

Determination of serum ALT and AST activity

Serum ALT and AST activity were determined by using spectrophotometer ALT and AST kit.

Determination of blood glucose

Blood glucose was determined by using glucometer (Kobayashi *et al.*, 1993).

Determination of serum malondialdehyde

Serum MDA was determined spectrophotometrically with a thiobarbituric acid (TBA) solution.

Statistical analysis

All data were expressed as means $\pm$ standard error (S.E) and statistical analysis was carried out using available statistical software (statistical package for social science (SPSS) version 11.5).

Data analysis was made using one-way analysis of variance (ANOVA). The comparisons among groups were done using Duncan post hoc test. P values <0.05 were considered significant.

Results

Pinealictomized rats showed a significant decrease in serum leptin level (34.307 $\pm$ 1.456 pg/ml) when compared with control (58.737 $\pm$ 0.992 pg/ml) and sham (55.619 $\pm$ 1.614 pg/ml) operated animals. Serum leptin level significantly increased (54.091 $\pm$ 0.727 pg/ml) in PINx animals provided with diet supplemented with MEL (60 mg/kg diet) when compared with PINx rats. Supplementation of control rats with MEL at a dose (60 mg/kg diet) significantly increased serum leptin level (77.747 $\pm$ 0.947 pg/ml). On the other hand, high dose of MEL (120 mg/kg diet) decreased serum leptin concentration (26.952 $\pm$ 2.744 pg/ml) when both groups compared with control animals (Table 1).

Serum testosterone level in PINx rats increased but not significantly as compared with control and sham animals. On the other hand, administration of MEL (60 mg/kg diet) to PINx rats decreased serum testosterone concentration non-significantly in comparison with PINx group. However, oral administration of MEL (60 mg/kg diet and 120 mg/kg diet) to control animals caused a significant decrease (1.952 ± 0.208 ng/ml) and (1.171 ± 0.124 ng/ml) respectively in serum testosterone concentration as compared with control rats (2.829 ± 0.408 ng/ml) (Table 1).

Statistical analysis revealed that PINx rats showed a significant increase in blood glucose concentration (113.833 ± 1.777 mmol/l) as compared with control and sham-operated surgery rats (90.000 ± 1.864 pg/ml) and (92.571 ± 2.147 pg/ml) respectively, while, oral administration of MEL (60 mg/kg diet) to PINx rats significantly ($P<0.05$) decreased blood glucose level (100.571 ± 1.394 mmol/l) in comparison with PINx animals. MEL (60 mg/kg diet) administration to control rats caused a non-significant decrease in blood glucose level, moreover, blood glucose concentration significantly decreased (79.833 ± 2.574 mmol/l) in control animals treated with MEL (120 mg/kg diet) when both groups compared with control group (Table 1).

Statistical analysis revealed that PINx animals showed a significant increase in serum MDA (2.352 ± 0.059 µmol/L) when compared with control (1.891 ± 0.091 µmol/L) and sham (1.944 ± 0.099 µmol/L) rats, while, PINx rats supplied with MEL (60 mg/kg diet) significantly showed reduced serum MDA level toward normal (1.966 ± 0.062 µmol/L) versus PINx group. Serum MDA level significantly decreased in normal rats treated with dietary MEL (60 and 120 mg/kg diet) (1.437 ± 0.144 µmol/L) and (1.432 ± 0.104 µmol/L) ,respectively, as both groups compared with control (Table 1).

Serum ALT activity was significantly increased in PINx rats (44.024 ± 0.835 IU/L) when compared with control (31.466 ± 0.984 IU/L) and sham-operated surgery (31.018 ± 1.088 IU/L) animals. Treatment of PINx rats with MEL (60 mg/kg diet) significantly reduced serum ALT activity toward normal value (31.652 ± 1.191 IU/L) versus PINx rats. However, there were significant reductions in serum ALT activity (22.327 ± 0.662 IU/L) and (20.088 ± 0.631 IU/L) in control animals when supplemented with MEL (60 and 120 mg/kg diet), respectively, as both groups compared with control (Table 1).

Pinealectomy significantly elevated serum AST activity (85.004 ± 0.987 IU/L) as compared with control (76.358 ± 1.483 IU/L) and sham (74.253 ± 0.784 IU/L) groups. MEL (60 mg/kg diet) administration to PINx rats decreased AST activity significantly toward normal value (74.456 ± 1.534 IU/L) in comparison to PINx group. Oral administration of MEL at doses (60 and 120 mg/kg diet) in control rats significantly ($P<0.01$) lowered serum AST activity to (63.782 ± 1.276 IU/L) and (60.807 ± 1.491 IU/L) respectively when both groups compared with the control (Table 1).

Discussion

Results of the current study showed that serum leptin level was significantly decreased in PINx rats, while, its level was increased after MEL (60 mg / kg diet) administration in PINx and control rats. Studies have reported contradictory results on the possible relationship between MEL and leptin secretion (Baltaci and Mogulkoc, 2007). Alonso-Vale *et al*., (2005) considered that MEL acts directly on adipocytes through G protein-coupled receptors MT1 and MT2, and the activation of these receptors might exert a positive modulation on leptin production by lowering cAMP levels.

On the other hand, Nieminen, (2000) demonstrated that in the autumn the shortening photoperiod increases the production of MEL in the pineal gland leading to weight gain and the storage of fat in the autumn, and this is reflected in the rising leptin levels.

Our study demonstrates that plasma leptin level of high dose of MEL group was significantly lower as compared with control groups. This result is supported by the findings of Rasmussen *et al*., (1999) that daily supplementation with high dose MEL decreased plasma leptin level. On the other hand, MEL is suggested to delay puberty (Kennaway and Rowe, 1997), whereas, leptin has been reported to have a permissive role in puberty onset (Gueorguiev *et al*., 2001). Furthermore, Reiter, (1998) suggested that there is an inverse interaction between plasma MEL levels and sexual maturation and that sexual maturation seems to be signaled by a decrease of MEL levels and an increase in leptin levels.

The current results show that serum testosterone level was decreased in MEL treated rats compared to controls, while, in PINx rats serum testosterone was significantly increased. This result is supported by Kus et al., (2002) finding that in PINx rats, serum testosterone levels were significantly increased comparing to sham-pinealectomized rats, while, daily MEL administration after pinealectomy resulted in a significant decrease in serum testosterone levels compared to levels in control and PINx rats.

Also, the current results are supported by the findings that MEL administration to control animals significantly decreased both luteinizing (LH) and testosterone level and that MEL inhibits testosterone secretion by acting at hypothalamo-pituitary axis. Furthermore, there is a functional relationship and feedback regulation between pineal gland and the testis (Yilmaz *et al*., 2000).

Besiseds, Valenti *et al*., (1997) demonstrated that MEL reduces LH-stimulated testosterone secretion by inhibiting adenylyl cyclase activity. Moreover, Valenti *et al*., (1999) concluded that MEL reduces gonadotropin releasing hormone(GnRH)-induced testosterone secretion by decreasing cytoplasmic calcium concentration, through impairment of the GnRH-dependent release of calcium from intracellular stores and blocking 17–20 desmolase enzymatic activity, an effect that occurs irrespective of changes in calcium concentration.

Pinealectomy significantly increased serum transaminases (AST and ALT), while, MEL (60 mg / kg diet) administration significantly decreased serum transaminases in both PINx and control rats. Also, high dose of MEL (120 mg / kg diet) decreased serum ALT and AST in control rats.

Dakshayani *et al*., (2005) found that administration of MEL restored the activities of AST and ALT to near normal values and that this effect of MEL is attributed to the hepatoprotective role of MEL. Furthermore, El-Sokkary *et al*., (2002) reported that the effect of MEL in maintaining normal hepatic and renal functions may be related to its ability to localize mainly in a superficial position in the lipid bilayer near the polar heads of membrane phospholipids.

On the other hand, Huang *et al*., (2009) reported that MEL treatment in rats in a dose-dependent manner, decreased liver and systemic oxidative stress, increased liver antioxidant activity. Also, Li *et al*., (2008) concluded that exogenous MEL protects liver by inhibiting the production of free radicals, reducing the concentration of tumor necrosis factor-α in systemic circulation and suppressing the expression of inter-cellular adhesion molecule 1 in liver. Also, it has been shown that liver is the final metabolic place of MEL and MEL protects liver from intestinal ischemia reperfusion injury (Xu *et al*., 2007) and studies have shown that MEL has no side effect when a high dose is used in rats (Pignone *et al*., 2006).

Results of the present study showed that pinealectomy significantly increased blood glucose, whereas, MEL reversed this effect toward normalization in PINx rats. In control rats treatment with MEL (120 mg / kg diet) caused a significant decrease in blood glucose. Similar finding has been demonstrated by Lima *et al*., (2001) recording that PINx animals showed a tendency to increased glucose and reduced insulin levels, and Prunet-Marcassus *et al*., (2003) found that blood glucose was significantly lowered without change in plasma insulin level when MEL was administered before lights-off.

Data of the present study show that pinealectomy significantly elevated serum MDA level in male albino rats, while, MEL administration to PINx and control rats significantly decreased the increased level of MDA. These results are consistent with Sahna *et al*., (2003) concluding that MDA levels resulting from ischemia reperfusion were significantly higher in the PINx rats than in the control group, and Mogulkoc *et al*., (2007) found that pinealectomy make the increase in MDA levels, while decreasing reduced glutathione(GSH) levels.

Results of the current study revealed that serum MDA level was decreased in animals receiving MEL (60 and 120 mg / kg diet). This result confirms that MEL decreases lipid peroxidation and is an effective antioxidant and free radical scavenger. Due to its small size and high lipophilicity, MEL can cross biological membranes easily and reach all compartments within the cell (Sener *et al*., 2003). Additionally, Hong *et al*., (2009) found that MEL protects DNA, proteins, and biological membrane lipids from the deleterious effects of free radicals. In conclusion: Melatonin has important roles in the control of leptin production and the regulation of reproductive system (by decreasing testosterone concentration) also ,it decreased serum transaminases in PINx rats and this suggests that melatonin has a hepatoprotective role.

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Table (1): Effect of melatonin on serum leptin, testosterone, blood glucose, ALT, AST and MDA in male albino rats

Parameters / Treatments	Serum leptin (pg/ml) **	Serum testosterone (ng/ml) *	Blood glucose (mg/dl) *	Serum MDA (µmol/L) **	Serum ALT (IU/L) **	Serum AST (IU/L) **
Control	58.73 ± 0.992 [c]	2.829 ± 0.408 [c]	90.00 ± 1.864 [b]	1.891 ± 0.091 [b]	31.466 ± 0.984 [b]	76.358 ± 1.483 [b]
Sham	55.61 ± 1.614 [c]	2.951 ± 0.158 [c]	92.57 ± 2.147 [b]	1.944 ± 0.099 [b]	31.018 ± 1.088 [b]	74.253 ± 0.784 [b]
PINx	34.30 ± 1.456 [b]	3.027 ± 0.32 [c]	113.83 ± 1.77 [d]	2.352 ± 0.059 [c]	44.024 ± 0.835 [c]	85.004 ± 0.987 [c]
PINx + MEL (60mg/kg diet)	54.09 ± 0.727 [c]	2.798 ± 0.223 [c]	100.57 ± 1.39 [c]	1.966 ± 0.062 [b]	31.652 ± 1.191 [b]	74.456 ± 1.534 [b]
Melatonin (60mg/kg diet)	77.74 ± 0.947 [d]	1.952 ± 0.208 [b]	87.16 ± 3.310 [b]	1.437 ± 0.144 [a]	22.327 ± 0.662 [a]	63.782 ± 1.276 [a]
Melatonin (120mg/kg diet)	26.95 ± 2.744 [a]	1.171 ± 0.124 [a]	79.83 ± 2.574 [a]	1.432 ± 0.104 [a]	20.088 ± 0.631 [a]	60.807 ± 1.491 [a]

Data presented as mean ± S.E

The same letters mean no statistical differences

The different letters mean statistical differences

****=P<0.01**

***=P<0.05**