Effect of exogenous melatonin on the secretion of testosterone concentration in rhesus monkeys (*Macaca mulatta*)

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Abstract:- **Background:** The impacts of melatonin (18 mg) were explored on testosterone concentration in male goat whereas testosterone amount was inspected in treated and untreated groups. It has been shown that the plasma FSH and LH concentrations increased on short days and decreased on long days, and that a corresponding cycle existed in the plasma concentration. The average value, basal level and numeral value of peaks of testosterone increased, by implantation of melatonin in rams.

Objectives: This study has been carried out on rhesus monkeys (*Macaca mulatta*) to examine the effect of exogenous melatonin on testosterone concentration (ng/mL).

Methodology: At the intervals of 15 minute sequential blood samples were collected in hepranized needles for a period of 2:45 hours and these samples were centrifuged at 3000rpm for 10 minute immediately. Melatonin single dose (5mg) given orally to four rhesus monkeys to examine the effect of melatonin on plasma testosterone concentration (ng/mL).

Results: The highest mean testosterone concentration was observed in the segment from 15 to 45 minutes and this changing (increase) in plasma testosterone concentration was not significantly higher compared to that of before treatment segment ($P>0.50$). Last segment 105 minutes to 120 minutes compared to segment 15 to 45 minutes showed highly considerable change (decrease) in plasma testosterone concentration ($P< 0.01$). After this there was gradual decrease in testosterone concentration from 5.14±2.27 to 3.95±1.39 ng/mL.

Conclusion: Present study supports that melatonin sharply increases in testosterone concentrations for short time and then starts decreasing.

Index Terms: Testosterone, Exogenous, Melatonin, *Macaca mulatta*, Concentration.

I. INTRODUCTION

Pineal gland has been recognized for more than 2000 years. About 50 years ago there was common belief about pineal gland that it is a rudimentary organ (Karasek and Winczyk 2006**)**. In 1958 condition has been altered when Aaron Lerner and colleagues has isolated pineal active substance that has been named as melatonin (N acetyl-5-methoxytryptamine) with its chemical structure (Lerner *et al*., 1958, 1959). After that numerous researchers started work to explore this mysterious compound. In late 70's after the organization of definite radioimmunoassay for melatonin interdisciplinary research carried out in the last four decades resulted into outstanding progress on the function of this hormone (Karasek and Winczyk 2006**)**. Melatonin is one of the most universally and widely distributed molecules in nature with efficient action present in one celled organisms, fungi, plants and animals along with mammals having same molecular structure (Pandi-Peruma *et al*., 2006, Karasek and Winczyk 2006).

Melatonin also manipulates other physiological tasks all the way through paracrine signaling (Pandi-Peruma *et al*., 2006, Vijayalaxmi *et al.*, 2002, Karasek 2006) due to its presence in various body parts like lymphocytes, skin, gastrointestinal tract and bone marrow. On the other hand, melatonin is synthesized naturally in most of the vertebrates (Kulczykowska 2002) including mammals (humans) in pineal gland and retina (Siu *et al*., 1998, 1999). Melatonin synthesized by the pineal gland in a

strictly nocturnal pattern (Sugden *et al*., 2004; Illnerova *et al.,* 1984, 1986). Melatonin plays vital role in various physiological activities like molting, deposition of fat, coat growth, circadian, hibernation (Arendt 1986, Lincoln 1991), seasonal responses, cardiovascular, immunological and behaviors (Zachmann *et al*., 1992, Boeuf *et al*., 2001, Pandi-Peruma *et al*., 2006). Along with above functions in the management of neurodegenerative diseases melatonin's cytoprotective properties have useful presumption (Reiter 1995a, Reiter *et al.,* 1996, 1998, Pandi-Peruma *et al.,* 2006).

Oncostatic and immune enhancing properties are also present in melatonin. Where as to treat the many circadian rhythm sleep disorders (shift-work sleep or jet lag disorder) chronobiotic properties have very important worth (Reiter 1995a, Turek and Gillette 2004, Pandi-Peruma *et al*., 2006). The jet-lag cause is possibly the most excellent clinical sign for melatonin exploit so far demonstrated (Reiter 1995b, Arendt and Deacon 1997, Cardinali *et al*., 2002). There are many symptoms together with impaired judgments and decision making, digestive upsets, sleepiness, memory lapses, irritability, apathy, lack of concentration, fatigue and headache caused by transcontinental flights during traveling. These are collectively known as jet-lag and those air travelers observe them appropriately who crossing several time zones because of stress to growing number of passengers. For ameliorating jet-lag symptoms the melatonin administration has been observed as helpful that specified by both controlled and uncontrolled studies (Reiter 1995a, Arendt and Deacon 1997, Cardinali *et al*., 2002, Turek and Gillette 2004). Particularly in depressive and elderly patients, to treat insomnia symptoms the sleep facilitating characteristics of melatonin plays vital role due to its internal sleep facilitator role (Pandi-Peruma *et al*., 2006**)**. The function of melatonin as a photoperiodical molecule for seasonal breeding has been documented in photoperiodical species in spite of the fact that its administrative impact in people and monkeys remains beneath examination (Pandi-Peruma *et al.,* 2006).

EFFECT OF MELATONIN ON TESTOSTERONE

Melatonin has been demonstrated to tweak in vitro discharge of testosterone by rodent (Ellis 1972, Ng and Lo 1988, Valenti et al., 1995) and human Leydig cells (Giusti et al., 1997). Undoubtedly, melatonin diminishes LH-stimulated testosterone emission by hindering adenylyl cyclase movement when it attaches to its pertussis-toxin-sensitive receptor (Valenti et al., 1997). The location of restraint of GnRH subordinate testosterone discharge and GnRH-induced changes in cytoplasmic calcium concentration ([Ca2+]i) was examined in grown-up rodent leydig cells refined in vitro by melatonin and found that melatonin hinders GnRH-dependent testosterone discharge, in portion by diminishing the cytoplasmic Ca2+ concentrations in rodent leydig cells refined in vitro. Melatonin blocked the testosterone emission actuated by GnRH and thapsigargin, which discharge Ca2+ particles from intracellular stores, and this inhibitory impact of melatonin was too watched within the nonappearance of extracellular calcium (Valenti et al., (1999). Testosterone production was diminished by melatonin in the availability of factors either of specifically actuating (PMA) or of hindering (staurosporine) protein kinase c (PKC) action (Foresta et al., 1995, Vanececk 1998). It has been inspected the impacts of melatonin management on serum hormone rhythms in a long-suffering male who needed perceptible circulating levels of endogenous melatonin management delivered vigorous nighttime crests in serum growth hormone and prolactin amount, whereas serum cortisol and testosterone were not impacted (Petterborg et al., 2003).

The impacts of melatonin (18 mg) were explored on testosterone concentration in male goat and testosterone amount was inspected in treated and untreated groups (Donmez et al., 2004). As compared to control group the average testosterone concentration shown as higher than the melatonin treated group. Though, the variation between melatonin treated group and control group was not considerable. A few researchers found that, depending on the dosage, melatonin implantation initiated a blood testosterone level enduring for days or indeed weeks (Kennaway and Gilmore, 1985, Lincoln and Ebling, 1985, Chemnieau et al., 1992, Kokalis et al., 2000). It has been shown that the

plasma FSH and LH concentrations increased on short days and decreased on long days, and that a corresponding cycle existed in the plasma concent (Lincoln and Mcneilly, 1989). The average value, basal level and numeral value of peaks of testosterone increased by implantation of melatonin in rams (Kokalis et al., 2000). Melatonin implants boosted the plasma testosterone concentrations in rams; this raise was more than two times greater than the control values acquired from rams (Rosa et al., 2000). This research has been carried out to explore the effect of exogenous melatonin on the secretion of testosterone concentration in adult male rhesus monkeys (*M. mulatta*).

II. MATERIALS AND METHODS

a. Animals utilized during study

In this research four adult male rhesus monkeys (*M. mulatta*) were consumed. The consumed monkeys were at the age of 8 to 10 years and they were assigned numbers as 0702, 0703, 0704 and 0705. Dental formula has been used to evaluate the age of these monkeys (Haigh and Scott, 1965). At the time of experiment the body weight has been measured and recorded as 6.8 to 10.5 kg. Separate cages have been used to house the monkeys in the Primate Facility of Quaid-i-Azam University, Islamabad in order to maintain them under standard colony environment. Fresh and clean fruits with vegetables were supplied following the normal circumstances of monkey food and water was present there at all times (ad-libitum).

b. Standardized feeding protocol

All monkeys were maintained under controlled conditions and hence one month prior to initiating of conduct experiment the appetite was monitored. Standardized feeding protocol has been followed that contains: fruits, boiled potatoes, eggs and bread with fixed time of 6:00am, 9:00am, 11:00am and 1:00pm respectively. Moreover, diet has been given to monkeys according to their weight of body.

c. Pharmacologic agents

In the present study two drugs were used as pharmacologic agents: Ketamine hydrochloride (Ketavet; Park-Davis, Berlin, FRG) and Melatonin (N-acetyl-5-methoxytryptamine) (5 mg).

d. Experimental protocol

During this study 04 monkeys were used to carry out the research. Bleeding started from -30 minute and melatonin was given orally at 0 minute (Figure 1).

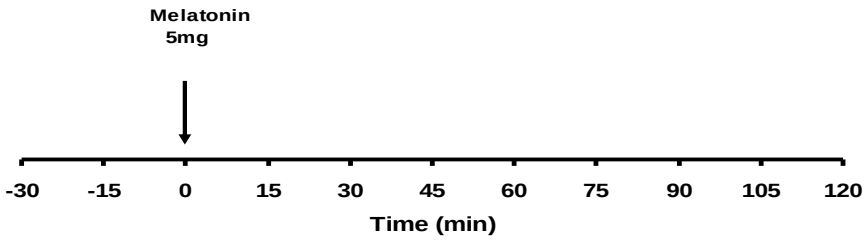

Fig 1. Experimental Protocol showing the bleeding time before and after melatonin treatment.

e. Chair restraining method

For chair restraining the monkeys were habituated for 8 weeks in advance of the experiment. On the chair for 2 hours in a day each monkey was restrained and these monkeys were also taught to take their food during this process. The monkeys were given ketamine hydrochloride (5mg/kg BW) and sedated for fixing or removing from the chair.

f. Catheterization

Prior to handling, ketamine (5 mg/kg; Ketavet, Parke-Davis, Freiburg, FRG) was used to anaesthetize monkeys. At the same time as in sedation, 60 minutes prior to initiation of sampling in the sephanous vein a Teflon cannula (Vasocan Brannule 0.8 mm/22 G.O.D., B. Braun, Melsangen AG, Belgium) was introduced in order to restrain the monkeys to the

chair. By the use of a butterfly tubing (20G and 300mm length) the free end of the cannula was fixed to a syringe. The monkeys were immobilized via the dose of ketamine that was enough for immobilized but not for to induce narcosis.

g. *Bleeding*

Prior to the single dose of melatonin (5mg) sequential blood samples were obtained at -30, -15 and 0 min (2.0 ml each after every 15 min). Later than melatonin administration in hepranized syringes the blood samples were achieved at 15, 30, 45, 60, 75, 90, 105, and 120 intervals. An equal volume of hepranized (5 IU/ml) saline was introduced into the tubing by subsequent extraction of each sample. During 09:00am to 10:00am all bleeding was conducted and immediate centrifugation of blood samples has been done for 10 min at 3000 rpm in order to separate and store at -15°C until analyzed.

h. *Statistical analyses*

Mean and standard error were calculated for the analysis of data and to compare the means student's t test was used. To show how testosterone concentrations regress on time later than treatment with melatonin the regression analysis of variance was used.

III. RESULTS

This research has been designed to examine the result of exogenous melatonin on testosterone concentrations in adult male rhesus monkeys. In the present study mean body weight (Mean ± SEM) of all the four monkeys (*M. mulatta*) is used (Table 1).

Table 1: Mean body weight (kg) of adult male rhesus monkeys (n= 4).

Monkey No.	Body weight (kg)
070	9.3
0703	5.7
0704	10.4
0705	9.1
Mean ± SEM	8.62 ± 1.05

EFFECT OF EXOGENOUS MELATONIN ON MEAN PLASMA TESTOSTERONE CONCENTRATION (ng/mL) IN ADULT MALE RHESUS MONKEYS (*M. MULATTA*).

Prior and later on than a single dose of 5mg of melatonin the plasma testosterone concentration (ng/ml) is shown (Table 2 and Figure 2). Four adult male rhesus monkeys were given melatonin treatment. Plasma testosterone concentration was recorded before and after treatment with melatonin. Mean concentration at -30 minutes for the four monkeys was 3.75 ± 2.05 ng/mL. There was a slight increase 4.01 ± 1.7ng/mL at -15 minutes. At 0 minute in plasma testosterone concentration decreased to 3.88 ± 1.74 ng/mL. At 0 minute 5mg of melatonin was administered orally to four monkeys. After 15 minutes of treatment testosterone concentration increased to 5.14 ± 2.27 ng/mL from that of at 0 minute. However this amplification in testosterone concentration was not considerably dissimilar from that at 0 minute ($t(6) = 0.28$; $P>0.7$). There was a slight decrease in testosterone concentration at 30 and 45 minutes time 4.69 ± 1.79 ng/mL, 4.12 ± 1.62 ng/mL respectively. Again at 60 minutes 4.21 ± 1.79 ng/mL, 75 minutes 4.25 ± 1.94 ng/mL, and 90 minutes time 4.68 ± 1.74ng/mL a gradual raise was noticed in plasma testosterone concentration. And whereas, the slight reduction was noticed at 105 minutes 4.21 ± 1.74 ng/mL, and 120 minutes 3.95 ± 1.3 ng/mL in plasma testosterone concentration.

TESTOSTERONE CONCENTRATION (ng/mL) AT VARIOUS SEGMENTS OF TREATMENT PERIOD.

Mean plasma testosterone concentration (Table 3) in the four monkeys before treatment in the segment -30 to 0 minute was 3.88 ± 1.24 ng/mL. The highest mean concentration of testosterone was observed in the segment from 15 to 45 minutes 4.65 ± 1.00 ng/mL and this amplification was not significantly higher compared to before treatment segment ($t(22)= 0.55$; $P>0.50$) in plasma testosterone concentration. In segment from 60 minutes to 90 minutes there was a very small decrease in plasma testosterone concentration 4.38 ± 0.95 ng/mL was not appreciable. In the next segment 105 minutes to 120 minutes decrease in mean concentration of plasma testosterone was 4.08 ± 1.03 ng/mL. This decrease

was not significant compared to segment 60 minutes to 90 minutes (t(18) = 0.15 ; P>0.80). Regression of testosterone concentration on time after treatment (b= - 0.28 ± 0.008) shows highly significant decrease in testosterone levels (F (1, 1) =1083, P<0.019) (Figure 3).

Table 2: Plasma Testosterone concentration prior and later than single dose (5mg) of melatonin administration in four rhesus monkeys (adult ♂ 04).

Time (min)	Plasma Testosterone Concentration (ng/mL)				Mean ± SEM
	Monkey No				
	0702	0703	0704	0705	
-30	9.56	0.92	3.73	0.77	3.75 ± 2.05
-15	8.94	1.12	3.88	2.10	4.01 ± 1.74
0	10.22	0.57	3.12	1.60	3.88 ± 2.17
15	11.59	1.24	4.84	2.87	5.14 ± 2.27
30	9.50	1.14	5.05	3.07	4.69 ± 1.79
45	8.50	0.89	4.42	2.68	4.12 ± 1.62
60	8.98	1.10	4.94	1.86	4.21 ± 1.79
75	9.86	1.33	3.87	1.96	4.25 ± 1.94
90	9.49	1.45	4.87	2.94	4.68 ± 1.74
105	8.96	1.13	4.65	2.12	4.21 ± 1.74
120	7.60	1.01	4.31	2.90	3.95 ± 1.39

Table 3: Plasma testosterone concentration (ng/mL) at different times prior and later on than single dose (5mg) of melatonin treatment in four rhesus monkeys (adult ♂ 04).

Time (min)	Prior to treatment	Later on to treatment		
	-30 to 0	15 to 45	60 to 90	105 to 120
Mean ± SEM	3.88 ± 1.24	4.65 ± 1.00	4.38 ± 0.95	4.08 ± 1.03

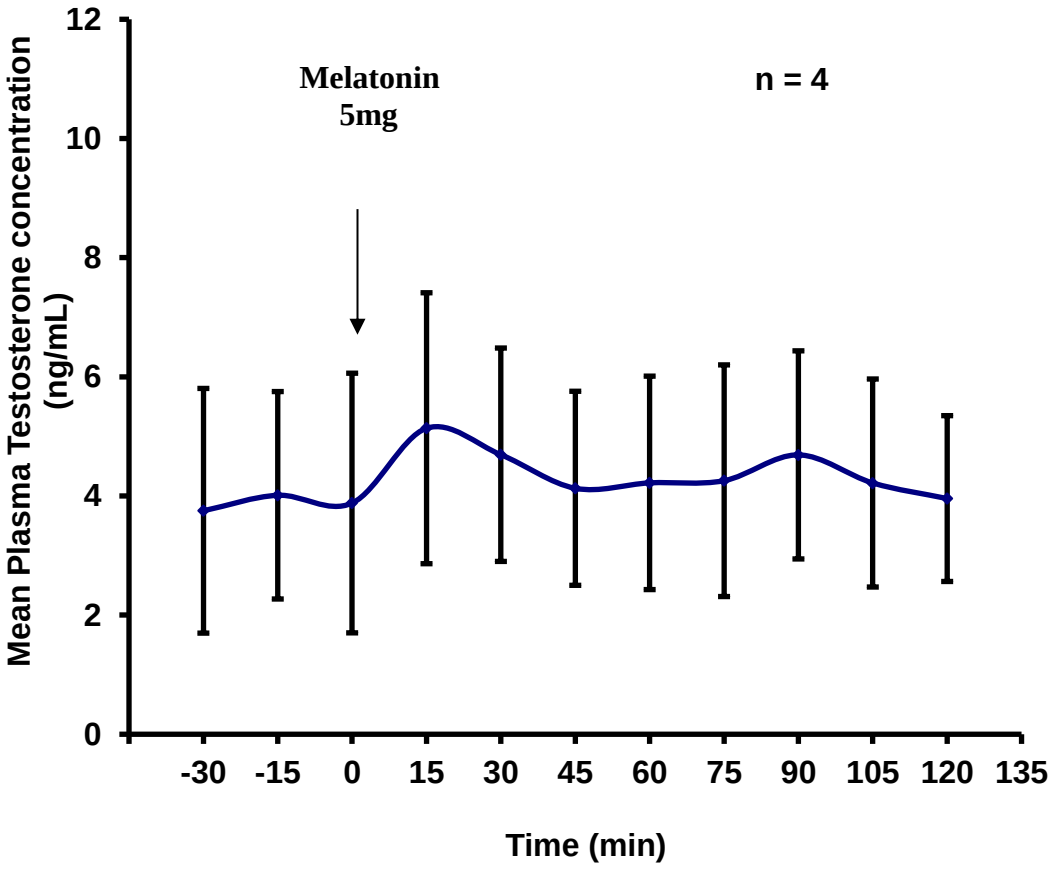

Fig 2. Change in mean (± SEM) plasma testosterone concentration (ng/mL) in adult rhesus monkey (n=4♂) prior and later to single dose of 5mg of melatonin administration.

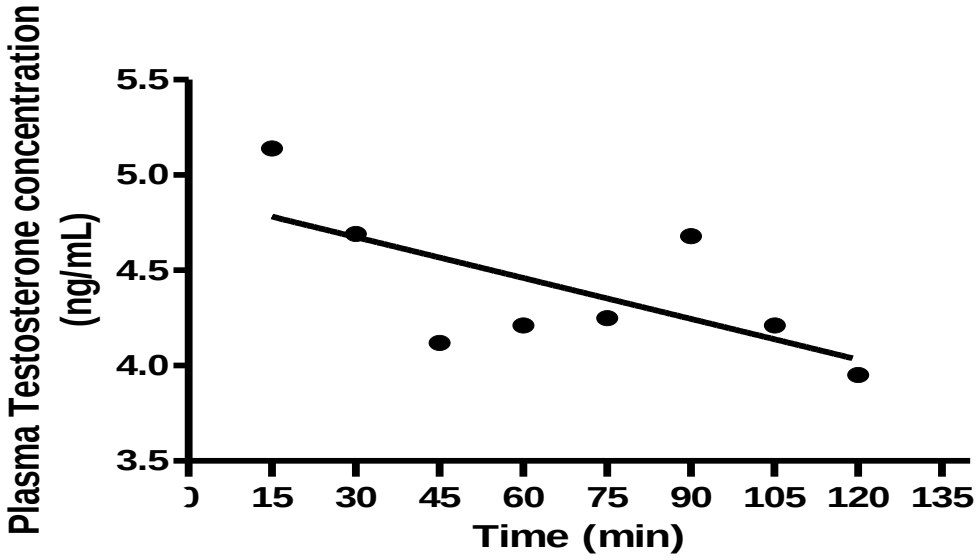

Fig 3. Calculated regression line against time after treatment indicating plasma testosterone concentration (ng/mL).

EFFECT OF EXOGENOUS MELATONIN IN ADULT MALE RHESUS MONKEYS (*M. MULATTA)* ON INDIVIDUAL PLASMA TESTOSTERONE CONCENTRATION (ng/mL).

Individual plasma testosterone concentration prior and later to single dose of melatonin (5mg) is shown (Table 3 and Figures 4 a, b). Four adult male rhesus monkeys were given melatonin treatment individually and plasma testosterone concentration was recorded prior and later to melatonin treatment. Individual plasma testosterone concentration of animals 0702, 0703, 0704, and 0705 at 0 minute timing was 10.22, 0.57, 3.12 and 1.60 (ng/mL) respectively. Melatonin was administered orally with a dose of 5mg to all four monkeys at 0 minute. After 15 minute of treatment there was a rapid increase in plasma testosterone concentration (ng/mL) to 11.59, 1.24, 4.84 and 2.87 (ng/mL) respectively, the level of plasma testosterone concentration (ng/mL) was observed upto 30 minutes in all individuals, then it decreased slowly at 45 minutes in all individuals to 8.50, 0.89, 4.42, and 2.68 respectively. Again it started increasing slowly in all individuals from 60 to 90 minutes and reached to 9.49, 1.45, 4.87, and 2.94(ng/mL) respectively. Once again testosterone concentration (ng/mL decreased from 105 to 120 minutes and reached to 7.60, 1.01, 4.31, and 2.90 (ng/mL) respectively.

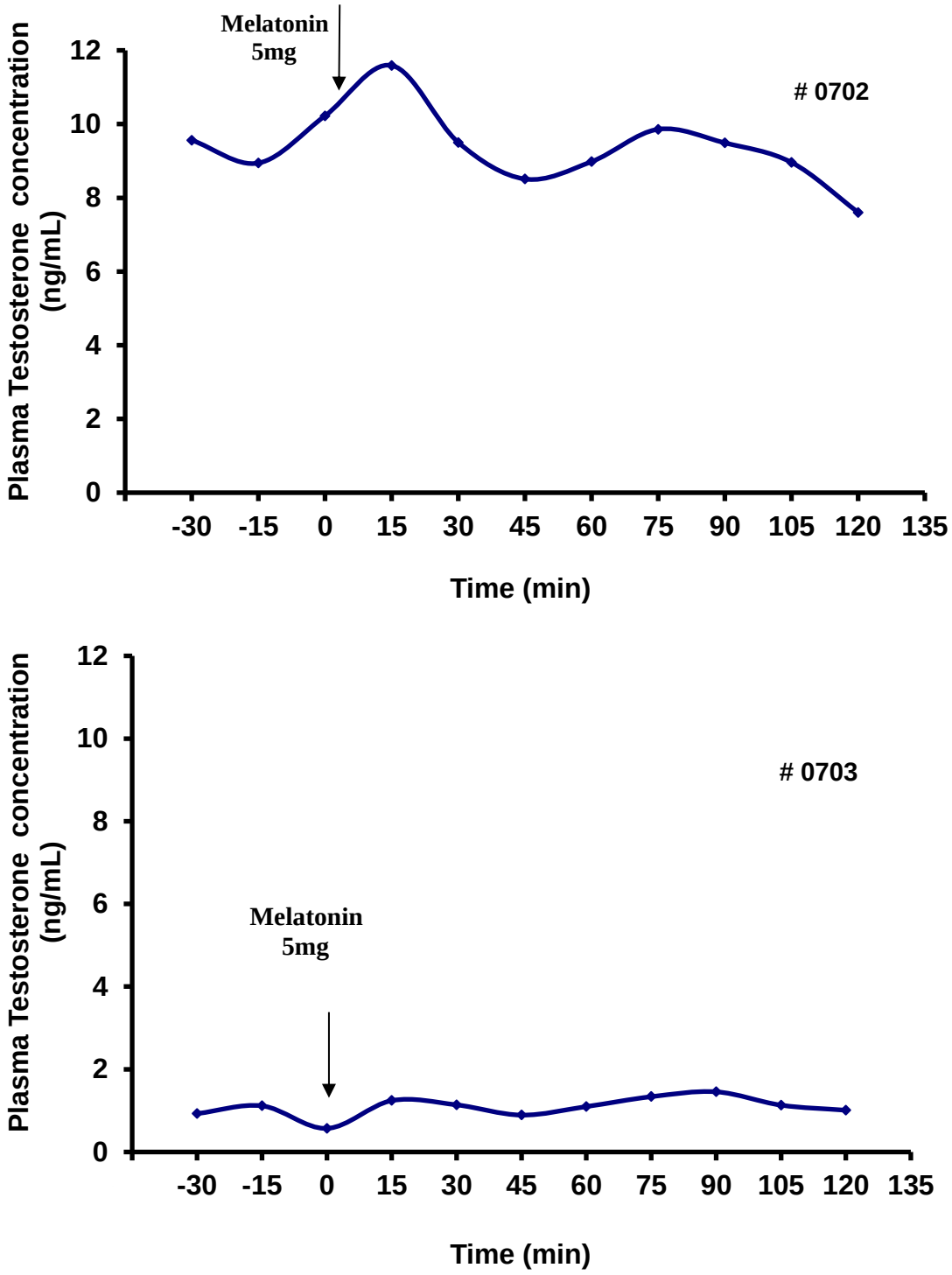

Fig 4 (a). Changes in individual plasma testosterone concentration (ng/mL) prior and later to melatonin treatment in adult rhesus monkey (♂04).

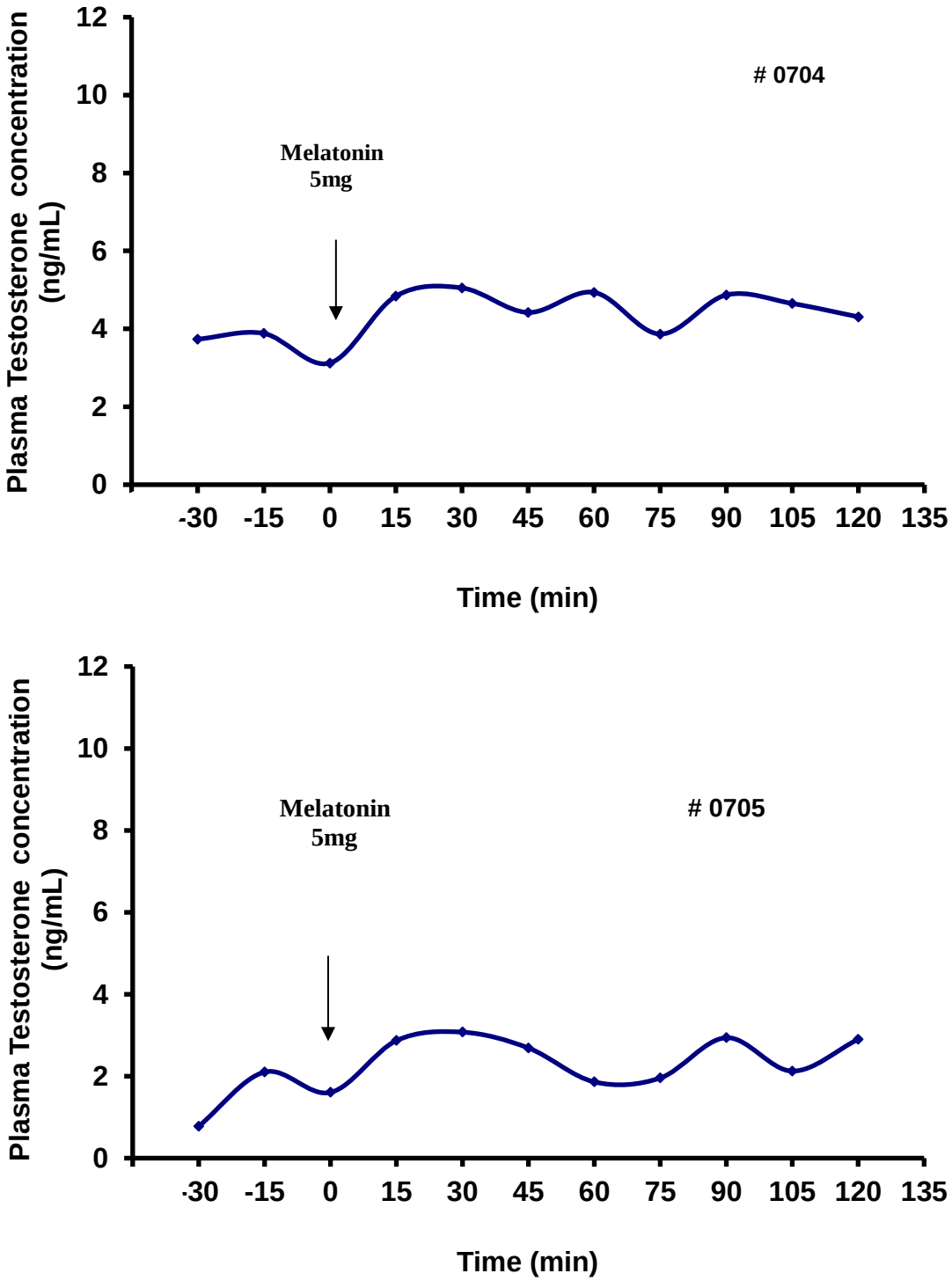

Fig 4 (b). Changes in individual plasma testosterone concentration (ng/mL) prior and later to melatonin treatment in adult rhesus monkey (♂04).

IV. DISCUSSIONS

This research has been conducted on adult *M. mulatta* (04 ♂) to uncover the effect of exogenous melatonin treatment on plasma testosterone concentration. To uncover the effect of exogenous melatonin (single dose of 5mg) on plasma testosterone concentration these four individuals has been consumed with mean body weight (8.62 ± 1.05).

In four adult male rhesus monkeys (*M. mulatta*) the effect of melatonin (5mg) on plasma testosterone concentration (ng/mL) has been explored also. A total of 11 blood samples after 15 minutes of interval were taken from 10:30 am to 2:30 pm and were analyzed by RIA. Before treatment mean basal testosterone concentration at -30 minutes 3.75 ± 2.05 ng/mL. Rizvi et al., (2000) reported 5.3 ± 0.7 ng/ml basal testosterone concentrations, whereas Goodman et al., (1974) reported serum basal testosterone concentrations 5.0 ± 0.8 ng/mL at 900 h and 17.0±1.5 ng/mL at 2100 h in monkeys weighing 6.4-11.9 kg.

Oral melatonin treatment caused a little insignificant amplification in mean plasma testosterone concentration as well as in individual monkeys after 15 minute. Mean plasma testosterone concentration before and after melatonin treatment was also observed in four segments of different timings. Before treatment from -30 to 0 minute testosterone concentration was found 3.88 ± 1.24 ng/mL. This concentration increased after melatonin treatment in 15 to 45 segment 4.65 ± 1.00 ng/mL but this was not a considerable amplify in plasma testosterone concentration as compare to before treatment segment ($P > 0.50$). in other segments from 60 to 90 minutes and from 105 to 120 minutes continuous decrease in plasma testosterone concentration was found, this gradual decrease in testosterone concentration after quick increase on time after treatment ($b = -0.28 \pm 0.008$) shows highly significant decrease in testosterone levels ($P<0.019$). This trend shows time dependant effect of melatonin on testosterone concentration (ng/mL).

The effects of melatonin on testosterone concentration in male goat have also been investigated by Donmez et al., (2004). They implanted 18mg of melatonin at the near base of ear subcutaneously in the test group animal. They found higher mean testosterone concentrations 1.45±0.34 in melatonin treated group as compared to control group 0.67±0.32ng/mL on 30th day of melatonin implantation. They also found increase in testosterone concentration 2.25±0.66 ng/mL in treated animals and 1.32±0.62 in control animals on 70th day of melatonin implantation. While, the variation between these two groups was not considerable. Present study also shows increase in testosterone concentration after acute oral melatonin treatments in monkeys but this was a not considerable amplify.

In past, some researchers observed that blood testosterone level amplified by melatonin implantation depending on the dose lasting for days or even weeks in ram lambs, sheep and goats (Kennaway and Gilmore, 1985, Lincoln and Ebling 1985, Chemnieau et al., 1992, Kokalis et al., 2000, Rosa et al., 2000) and this amplification was found as more than double as superior as the control values acquired from rams. Where as in this study 5mg melatonin has increased plasma testosterone concentration for a short time in male rhesus monkeys and that was a very small increase. Like present study, it has been suggested that in male coreole goats plasma testosterone concentration elevated by melatonin implantation (Delgadillo et al., 2001).

In male patients who lacked noticeable circulating levels of endogenous melatonin the effect of exogenous melatonin administration on serum testosterone rhythm was studied and in testosterone no any change detected (Petterborg et al., 2003). In the leydig cells of human (Giustina et al., 1997) and rat (Valenti et al., 1995, Ellis 1972, Ng and Lo 1988) in vitro secretion of testosterone has been modulated directly by melatonin. Whereas present study have shown plasma testosterone increase in monkeys after melatonin administration.

In male and female rat pus during the investigation of prenatal melatonin exposure on testosterone secretion it was found that dihydrotestosterone concentrations and plasma testosterone has been condensed (Okatani et al., 2001), While it has been noticed that the testicular testosterone content of offspring amplified by maternal subcutaneous administration of

melatonin (25 mg per day) on daily basis. Additional former research recommended that during the premature life in male offspring testicular function influenced by the maternal pineal gland (Jarrige et al., 1990, 1992). The influence of melatonin on testosterone concentration has been examined in the leydig cells of adult rat cultured in vitro and observed decline in concentration of testosterone (Valenti et al., 1999). Melatonin is intensely concerned in the check and balance of androgen discharge by isolated leydig cells from rat testis. This process may happen in humans and monkeys also in vivo. According earlier research carried out in pubertal boys, it has been investigated that during the night time circulating melatonin is higher in level and whereas testosterone highest in level during the early morning (Reichlin 1989), the same might be possible in rhesus monkeys.

V. CONCLUSION

In conclusion, present study supports that melatonin sharply increases in testosterone concentrations for short time and then starts decreasing. Consequently results in significant decrease in testosterone concentrations with increase in time. Thus it shows the time dependent effect of melatonin on testosterone concentrations.

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