Zagazig J. Pharm. Sci, Dec. 1996,
Vol. 5, No. 2, pp 84-91

ISSN 1110-5089

THE INFLUENCE OF CHRONIC MELATONIN TREATMENT ON GONADOTROPINS, SEX HORMONES AND LIVER AND KIDNEY FUNCTIONS IN ADULT MALE AND FEMALE RATS

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ABSTRACT

The present study aimed at demonstrating the possible changes in gonadotropins, sex hormones as well as liver and kidney functions induced by chronic administration of melatonin (Mel) 300µg/100g body weight S.C. daily for two months to intact adult male and female rats. Chronic injection of Mel. caused a significant elevation in serum luteinzing hormone (LH) and follicle stimulating hormone (FSH), but significantly reduced serum testosterone (T) level in male rats when compared with control group. In female rats administration of Mel. markedly reduced serum LH, estrogen (E) and progesterone (P) levels, but failed to change serum FSH as compared with control group. Subcutaneous injection of Mel. induced a significant elevation in serum AST level in both male and female rats. A marked reduction was also induced in serum urea level of male rats when compared with control group. The results suggest that chronic use of Mel. in rats, of either sexes, had an inhibitory effect on the reproductive system. The antireproductive effect of Mel. is due to reduction in serum T. level of male rats, and the reduction in P and E levels of female rats. This inhibition in ovarian function induced by Mel. in female rats may suggest the possible use of it as contraceptive alone or together with common contraceptives. We can also conclude that chronic administration of Mel. in rats affects liver function by elevating serum AST level, and alters kidney function, in male rats only, by reducing serum urea level.

INTRODUCTION

Melatonin is a putative pineal hormone received special interest in the recent years, especially as component in the overall control of the reproductive axis. Inhibitory influence of Mel. in human reproduction have been suggested by indirect evidences showing an association among defective ovulation or anovulation and an elevation in the circulating levels of Mel.[1,2,3]. In patients with oligozoospermia and azoospermia an elevation in Mel. levels was observed, levels of FSH, LH and prolactin were elevated in infertile patients. The possibility that an increase in Mel. concentration is either the primary feature that leads to the regression of the seminiferous epithelium or is secondary and depends on elevated gonadotropins and/or prolactin levels[4]. It was reported that there was a decrease in Mel. level preceded FSH increase during perimenopause suggesting that Mel. may be permissively linked to the initiation of menopause[5].

The pineal hormone Mel. is thought to modulate the effect of the pineal gland on seasonal reproduction by altering the release of gonadotropins. The pituitary gland undergoes changes in sensitivity to gonadotropin releasing hormone (GnRH) during sexual maturation and the pineal gland may play a role in this process[6].

In vitro studies revealed that Mel. has a direct stimulant effect on ovine granulosa cells to produce progesterone. However inconclusive results were obtained by studies evaluating the effect of exogenous Mel. on reproductive parameter and in particular on gonadotropin release[8-10].

The presence of Mel. receptors in the chicken kidney[11] and the possible regulation and second messenger system of Mel. receptors in CNS and peripheral tissues suggested the possible involvement of Mel. in biological changes in the renal and other systems and organs like the liver.

The present study was designed to investigate the possible modulatory effect of chronically administered Mel. on the gonadotropin serum levels as well as the gonadal steroids of male and female rats. The study was also extended to evaluate the chronic action of Mel. on the liver and kidney functions of male and female rats.

MATERIALS AND METHODS

Animals:

Twenty four male and twenty four female rats weighing 150-200gm at the beginning of the experiment, in January, were used. The animals were housed under 12 hr. light/dark cycle at temperature of approximately 23°C, provided with food and tap water ad libitum.

Experimental Design:

The animals were divided into eight groups four males and four females each consisting of six rats. Two groups of each sex were used as control, one for measurement of control gonadotropins and sex hormones and the other was used as control for measurement of parameters of liver and kidney functions. The other groups were treated by Mel. 300µg/100g (Sigma Chem. Co.) subcutaneously daily between 17.00 and 18.00hr for two months.

Fresh Mel. solution was prepared daily by dissolving Mel. in a minimum of absolute ethanol and diluted in 0.9% Nacl. The day after the last injection blood was drawn from the orbital sinus of all groups, and centrifuged, the serum was stored frozen at 20°C until assayed for hormones and parameters of liver and kidney functions.

METHODS

1- Estimation of serum gonadotropins:

Serum LH and FSH levels were measured by specific kits (DELFIA) Pharmacia Company Finland.

2- Estimation of serum sex hormones:

Serum testosterone level was estimated by a radioimmunoassay method following Yallow and Berson[12].

Serum E and P levels were also measured using specific kits (DELFIA) Pharmacia Company Finland.

3- Investigation of liver function:

a- Determination AST and ALT:

Serum levels of AST and ALT were estimated colourimetrically following the method of Reitman and Frankel[13].

b- Determination of serum bilirubin level:

Bilirubin level in serum was estimated colourimetrically according to the method of Jendrassi and Grof[14].

c- Estimation of serum albumin level:

Serum albumin level was determined following Rodkey[15] and modified by Doumas and Biggs[16].

d- Estimation of total protein:

Serum total protein level was estimated colourimetrically[16].

e- Estimation of alkaline phosphates level:

Alkaline phosphates level was estimated[17].

4) Investigation of the kidney function:

a) Determination of serum urea level:

According to the method reported by Fawcett and Scott[18] serum urea level was estimated colourimetrically.

b) Determination of serum creatinine level.

It was determined following the method described by Husdan and Rapoport[19].

Statistical analysis:

Results are expressed as means ± SE. Unpaired Student's T-test was used to assess significance, $P < 0.05$ was considered to be significant[20].

RESULTS

A- Effect of Mel. on male rats:

1- Effect on serum gonadotropins:

Administration of Mel. (300µg/100g) daily for two months produced significant elevation of serum LH

Zagazig J. Pharm. Sci, Dec. 1996,
Vol. 5, No. 2, pp 84-91

ISSN 110-5089

from 5.2 ± 0.13 in the control group to (7.4 ± 0.32)µg/l. The serum level of FSH was markedly increased from (4.3 ± 0.21) to (6.8 ± 0.10)µg/l. Figure (1 A & B).

2- Effect on serum sex hormones:

Serum testosterone level was significantly reduced from 5.21 ± 0.03 in the control group to (3.43 ± 0.024 ng/ml in the treated group Fig. (2 A).

3- Effect on liver function:

Chronic administration of Mel. caused a marked elevation in serum AST level from (39.26 ± 1.03) in the control group to (71 ± 1.8)u/l in the treated group. The other liver parameters were not altered by Mel. Table (1).

4- Effect on the kidney function:

A significant reduction in the serum urea level was induced in the Mel. treated group when compared with control group (34.3 ± 0.11), (19.2 ± 0.69) mg/dl. Serum creatinin level was not affected by Mel. treatment. Table (2).

B- Effect of Mel. on female rats.

1- Effect on serum gonadotropins:

Chronic administration of Mel. to female rats induced a significant reduction in serum LH level to reach (2.6 ± 0.01) µg/l when compared with the control value (4.6 ± 0.23) µg/l. Injection of Mel. failed to alter serum FSH level in female rats. Figure (1 C & D).

2- Effect of Mel. on sex hormones:

The serum level of progesterone of control group was (15.5 ± 0.81)µg/l, this value was significantly lowered in the Mel. treated group to reach (9.8 ± 0.153) µg/l. Melatonin caused a significant reduction in the serum estrogen level from(27.3 ± 1.51) ng/l in the control group to (19.4 ± 1.35) ng/l in the treated group Fig. (2 B & C).

3- Effect on liver function:

Melatonin injection produced a significant elevation in serum AST level, but did not cause any change in the other parameters under investigation. Table (1).

4- Effect on kidney function:

Chronic administration of Mel. for two months caused a non significant effect in both urea and creatinin serum level of female rats. Table (2).

Table (1): Effect of melatonin (300µg/100g) daily for two months on liver function of female and male rats.

	Female rats		Male rats	
	Control group	Treated group	Control group	Treated group
AST (u/l)	36.4±15.1	69.9±1.688	39.26±1.03	71.00±1.8*
ALT (u/l)	18.7±0.63	19.2±0.41	19.8±0.43	20.6±6.7
Albumin (mg/dl)	4.1±0.13	4.52±0.15	4.9±0.12	4.78±0.134
Total Protein(mg/dl)	7.8±0.31	8.46±0.30	8.5±0.53	8.16±0.204
Bilirubin (mg/dl)	0.85±0.023	0.824±0.021	0.87±0.042	0.902±0.029
Alkaline phosphatase (u/dl)	20.1±0.015	19.00±0.47	22.8±1.01	23.2±0.84

Values are expressed as mean ± SE
* Significantly different from control group at $P < 0.05$

Table (2): Effect of melatonin (300µg/100g) daily for two months on kidney function of female and male rats.

	Female rats		Male rats	
	Control group	Treated group	Control group	Treated group
Urea (mg/dl)	35.2±1.06	34±1.05	34.3±0.11	19.2±0.69*
Creatinin (mg/dl)	0.92±0.01	1.09±0.035	0.81±0.01	0.696±0.02

Values are expressed as mean ± SE
* Significantly different from control group at $P < 0.05$

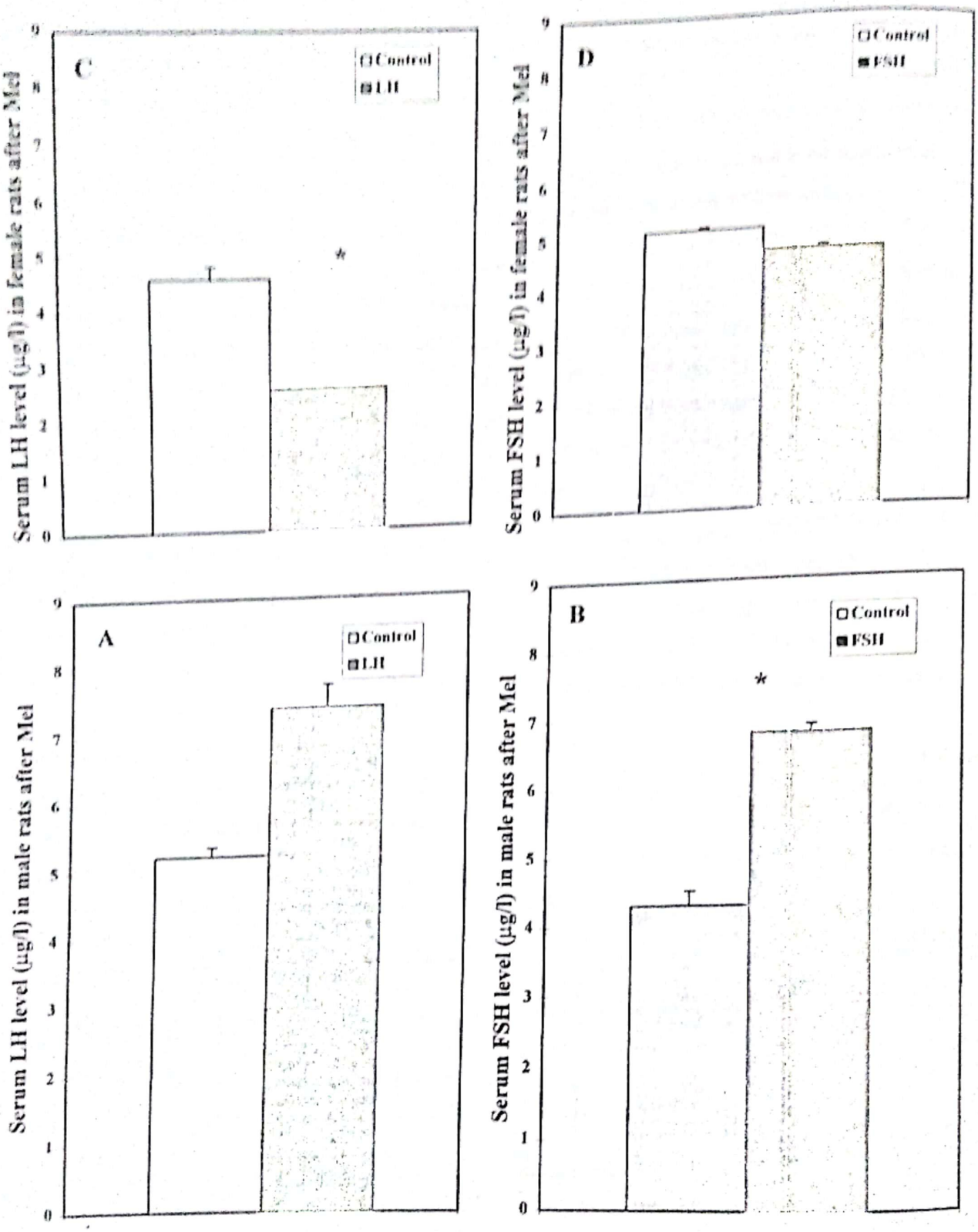

Figure (1): Effect of melatonin (300 µg/100g) daily for two months on serum gonadotropins of male and female rats.

* Significantly different from control group at $P < 0.05$.

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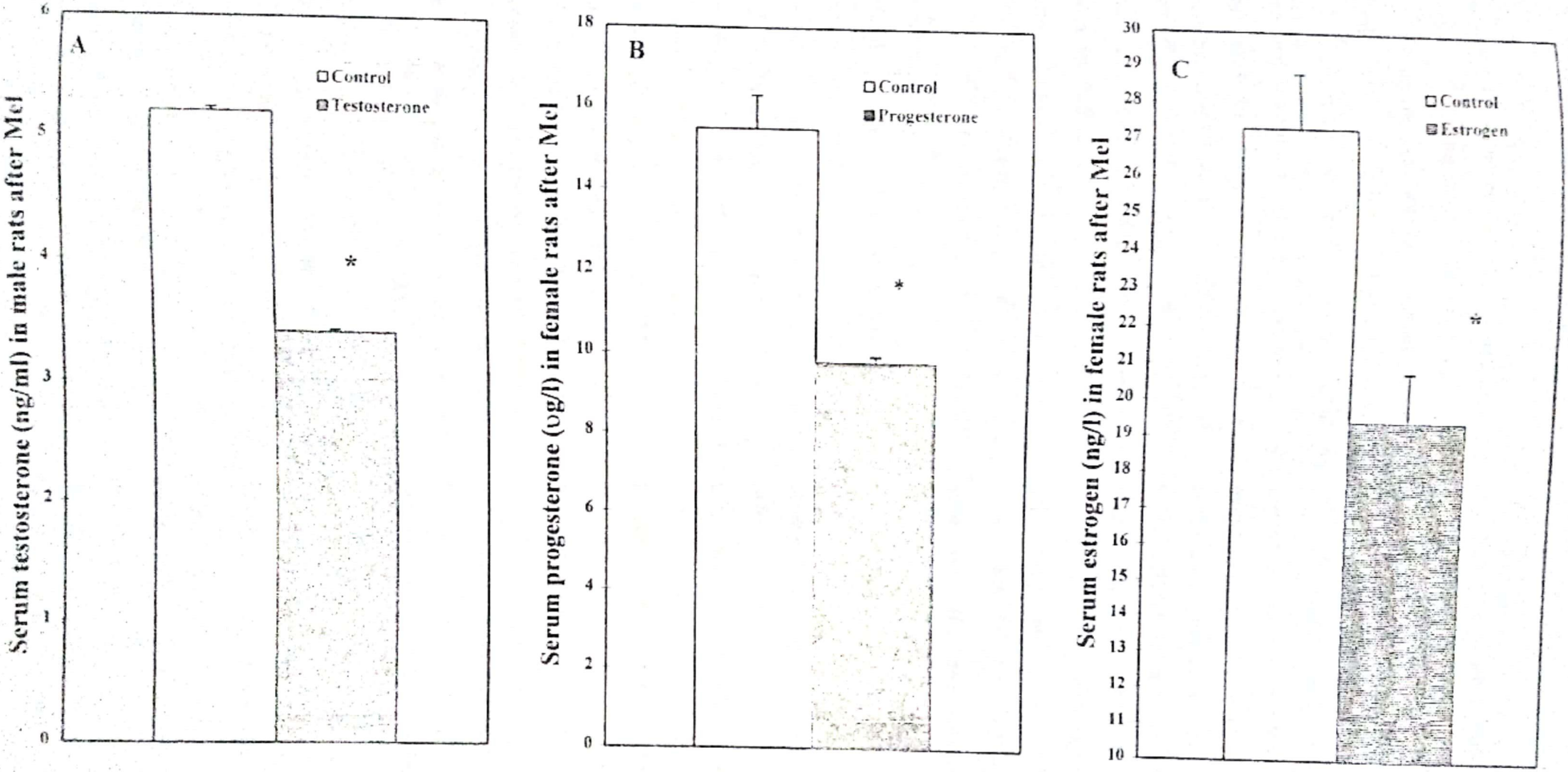

Figure (2): Effect of melatonin (300 µg/100g) daily for two months on serum sex hormones of female and male rats.

* Significantly different from control group at $P < 0.05$.

DISCUSSION

The present study revealed that s.c administration of Mel. for two months in male rats caused a significant elevation of LH and reduction of serum T. levels. These findings agree with the previous results[21] which demonstrated that Mel. not only failed to stimulate but provided an additional inhibitory effect on reproductive system in male rats. The increase in serum LH and FSH in the present work also agree with the results of Ntoumi and Co-Workers[22] who found that in intact mink Mel. induced a significant increase in FSH and LH concentration after 3 and 8 weeks suggesting stimulation of gonadotropin releasing hormone release.

It was reported that serum LH levels were decreased in the Mel. treated male rats on day 30, but elevated on days 45 and 60 of age as compared to controls. Neonatal Mel. administration induced an ealier sexual maturation in male rats, possibly related to prolactin, LH, MAO and phenylethanolamine N-methyl treansferase[23]. Melatonin injection in male rats significantly decreased serum testosterone level, suppressed spermatogenesis and reduced the weight of testis and accessory sex organs[24, 25]. The absence of correlation between serum gonadotropins and testosterone in the present study may suggest a direct action of Mel. on Male gonads and consequently the possible presence of testicular Mel. receptors. In vitro study on mouse leydig cells added an additional support to the above suggestion, since it demonstrated that Mel. exerts its remarkable antigonadotrpic effect, at least in part, through the direct decrease of testosterone production[26].

In the present investigation, serum LH, P and E. levels were significantly reduced, while FSH was not affected after s.c. injection of Mel. daily for two months. In a study on intact female hamsters Mel. injection caused significant reduction in serum level of FSH, LH, prolactin, uterin and pituitary weights after 8 weeks of treatment[27] suggesting that Mel. may exert its antireproductive effects by modulating estrogen receptor levels in medial preoptic area neurons, thus influencing steroid feed back mechanism.

Our results are in accordance with the previously reports that after a period of 4 months, daily administration of Mel. caused significant decrease in LH, P and E serum levels in Women[10]. The above authors suggested that Mel. alone and in combination with progestin inhibit ovarian function in women. Melatonin can modulate gonadotropin secretion by acting on a dopamin mechanism independent of hypothalamic suprachiasmatic areas[28].

Melatonin is thought to mediate the effect of pineal gland on seasonal reproduction by altering the release of gonadotropins. The highest concentration of Mel. receptors was found in the pars tuberalis where pituitary hormones, in particularly LH, have been localized in mammals[29]. The above authors reported that Mel. acts on its receptors in the pars uberalis to inhibit LH release. This explains the significant reduction in serum LH and the non significant effect of Mel. on FSH in the present study. Vanecek and Klein[30] observed that Mel. inhibits GnRH stimulation of Ca^{++} influx in neonatal rat gonadotophs and this probably explains the inhibitory action of Mel. on GnRH stimulation of LH release.

On the other hand in vitro study to determine the effect of Mel. on steroid hormone production by ovine granulosa and luteal cells revealed that Mel. stimulates granulosa cells to produce P. (5).

Our results are not in accordance with those obtained by Trentini et al.[31] who suggested that the age-related decrease in circulating Mel. during the night in female rats may contribute to the reproductive

ISSN 1110-5089

in female rats may contribute to the reproductive decline of aging and that this effect may involve the central opoid system.

The present data revealed a significant elevation in serum AST level in both male and female rats after chronic Mel. injection. This indicates an alteration in liver function in the used dose level for two months.

It was also found that the same treatment caused a significant reduction in serum urea level of male rats only. The kidney Mel. receptors may modulate the adenylate cyclase leading to the biological responses in the renal system[32]. the reduction in urea level in male rats together with the elevation in serum LH & reduction in T. agree with the results obtained by Elias et al.[33] who proved an association between nephrotic disorders and hypoandrogenism with significant elevation of serum LH hormone of male rats.

In conclusion, chronic administration of Mel. caused inhibition of the reproductive system through reduction of T. in male and E and P in female rats. The liver function of both male and female rats was altered through elevation of serum AST level. The Kidney function of male rats was changed by reduction of serum urea level.

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التأثير المزمن للعلاج بالملاتونين على الجونادوتروبينات والهرمونات الجنسية ووظائف الكبد والكلى فى ذكور واناث الفئران البالغة

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تهدف هذه الدراسة إلى تعيين التغيرات المحتملة فى الجونا دورتروبينات والهرمونات الجنسية ووظائف الكبد والكلى الناتجة عن الاستخدام المزمن للملاتوتين (٣٠٠ ميكروجرام/١٠٠جم) والمحقون تحت الجلد يومياً لمدة شهرين فى ذكور وأناث الفئران البالغة. وقد وجد أن الحقن المزمن للملاتونين يسبب ارتفاعاً واضحاً فى نسبة هرمونات FSH,LH والتستوستيرون فى مصل دم ذكور الفئران إذا ما قورنت بالمجموعة الضابطة. وتبين من البحث أن حقن الملاتونين فى إناث الفئران يسبب انخفاضاً ملحوظاً فى مستوى هرمونات LH والا ستروجين والبروجستيرون فى مصل الدم وبينما لم يتأثر مستوى هرمون FSH بالمقارنة مع المجموعة الضابطة أدى حقن الملاتونين لمدة شهرين إلى زيادة نسبة انزيم الكبد الاسبرتات ترانس اميناز فى مصل دم كل من إناث وذكور الفئران وانخفاض نسبة البولينا بدرجة ملحوظة فى الذكور فقط.

مما سبق نستنتج أن الاستخدام المزمن للملاتونين فى الفئران له تأثير مثبط للجهاز التناسلى ناتج عن خفض مستوى التستوسيرون فى الذكور ومستوى هرمونى الاستروجين البروجستيرون فى الاناث هذا الانخفاض فى وظيفة المبيض فى الاناث الناتج عن الملاتونين بالجرعة المستخدمة يدعو إلى احتمال نجاح استخدامه لمنع الحمل إما منفرداً او مع هرمون البروجستيرون.

ويمكننا ايضاً أن نستنتج ان استخدام الملاتونين المزمن قد أدى إلى حدوث اضطراب فى وظائف الكبد ممثلة فى رفع نسبة الأسبرتات ترانس اميناز فى كبد ذكور واناث الفئران وتغير فى وظائف الكلى فى الذكور فقط عن طريق خفض مستوى البولينا فى مصل الدم.