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Melatonin negatively regulates fertility: A study in albino rat *(Rattus norvegicus)*

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Summary

This study was performed to evaluate the biological activity of melatonin on the fertility of male and female albino rat. The tested dose (1.0 mg/kg) of melatonin and reference drug "primovlar" were administered through oral route daily to female rats 2 cycles before mating (group I) and from the first day of mating till 21 days (group II). On the other hand, the male rats were administered only the tested dose of melatonin for two weeks before mating (group III). The effects of melatonin and primovlar administration on fertility index, pregnancy percentage, sperm count, hormonal levels (prolactin, FSH, LH, progesterone and estradiol) and some biochemical parameters (total lipid and cholesterol) were investigated. Also, histopathological changes of ovaries and testes of rats treated with melatonin were examined. The results showed significant decrease in the pregnancy percentage and fertility index and non-significant decrease in the sperm count after melatonin treatment. The data also showed significant increase in prolactin level and significant decrease of FSH, LH, estradiol and progesterone levels of male and female albino rats treated with melatonin or primovlar versus those of controls. In the same domain, biochemical analysis revealed significant elevation in total lipid and cholesterol levels. Histological examination showed little changes in ovarian and testicular structure in melatonin-administered animals versus those of controls.

In conclusion, the tested dose of melatonin affected the fertility in negative manner and exerted a more potent effect than primovlar.

Key words: Albino rat, fertility, melatonin, primovlar

Introduction

Melatonin is a naturally occurring compound found in animals, plants, and microbes (Reiter et al., 2002; Paredes et al., 2009). In most vertebrates including human, melatonin is secreted primarily from the pinealocytes in the pineal gland located in the brain (Boutin et al., 2005). Pinealocytes function as neuroendocrine transducers to secrete melatonin during the dark phase of light / dark cycle and, consequently, melatonin is often called the hormone of darkness (Grivas et al., 2007)). Synthesis of melatonin also occurs in other areas of the body including retina, gastrointestinal tract, skin, bone marrow, lymphocytes, platelets and thymus from which it may influence other physiological functions through paracrine signaling (Srinivasan et al., 2009a, b).

Melatonin acts like an internal synchronizer and, thus, it has a regulatory effect on a variety of physiological functions including circadian rhythm, blood pressure, oncogenesis, retinal physiology and osteoblast differentiation (Witt-Enderby et al., 2003; Arendt, 2006; Reiter and Korkmaz, 2008). It also has antitumor activity (Vinogradova et al., 2008) and it is an effective antioxidant, which scavenges free radicals and up-regulates several antioxidant enzymes (Jou et al., 2010).

Several studies reported that melatonin plays important role in reproduction and aging. There is an association between endogenous melatonin level and onset of puberty. The pineal gland is large in children but shrinks at puberty, so it plays major role in sexual development. The high melatonin level in children is believed to inhibit sexual development until onset of puberty when melatonin production is reduced (Reiter, 1998; Macchi et al., 2004)

There is direct correlation between melatonin secretion and menstrual cycle (Barron, 2007). Melatonin reaches its Zenith during menstruation and its nadir during ovulation (Presl, 1993). Melatonin levels decrease during aging and attains minimum levels at menopause (Bellipanni et al., 2005; Mal'Steva et al., 2007).

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Also, there are indications that abnormally elevated endogenous melatonin levels and pharmacological doses of melatonin have antigonadal effects in humans and some animals (Srinivasan et al., 2009a, b). In female rats, which like human are not seasonal breeders, large doses of exogenous melatonin completely inhibited ovulation and prevented LH surge when administered during the critical period of proestrous (Turek et al., 1987).

In humans, increased plasma concentration of melatonin was repeatedly found in women suffering functional hypothalamic amenorrhea; intravenous administration of large doses of estrogen reduced nocturnal melatonin (Boczek-Leszczyk et al., 2007). Also, women with exercise-induced amenorrhea had melatonin level twice as high as in those with normal cycles (Walker et al., 1996). Pharmacological doses of exogenous melatonin given to young healthy woman (daily oral 300 mg for 4 months) altered ovarian activity and partially inhibited ovulation (Brzezinski, 1997). Administration of 3 mg per day of melatonin for 3 months resulted in marked decline in sperm count and a decline in sperm quality in healthy young men (Luboshitzky et al., 2002). The antigonadal effect of melatonin raised the possibility of a caused relationship between high melatonin concentration and suppressed hypothalamic - pituitary - gonadal axis in humans (Brzezinski, 1997). Also, it has been suggested that melatonin would exert an effect on human reproduction by directly modulating ovarian function (Woom et al., 2001) and spermatogenesis (Wojtowicz & Jakiel, 2002).

In contrast to the previous studies, Leibenluft et al. (1994) found that melatonin does not appear to vary systemically over the course of menstrual cycle, and the endogenous levels of estradiol, progesterone and prolactin do not affect the levels of the circadian phase of melatonin secretion. Also, a research team in Italy has found that melatonin supplementation in the evening in the perimenopausal women produces an improvement in thyroid function and gonadotropin levels, as well as restoring fertility and menstruation and preventing depression associated with menopause (Bellipanni et al., 2005). Also, Luboshitzky et al. (2000) reported that long-term melatonin administration does not alter secretory patterns of reproductive hormones in normal men. In addition, Dair et al. (2008) suggested that in non-photoperiodic animals such as rats, melatonin may positively affect the endometrial morphology and improve embryo implantation. Melatonin may also play role in other aspects of human reproduction such as protection of fetus from oxidative process and beneficial effect on the outcome of vitro fertilization (Reiter et al., 2009).

Thus, the previously studies yielded conflicting results on the effect of melatonin on reproduction. So, this work was designed to investigate if melatonin affects fertility in albino rats in negative or positive manner and to clarify its mode of action in comparison to the effect of primovlar, one of the most widely used oral contraceptive.

Materials and Methods

Animals

Male and female albino rats (*Rattus norvegicus*) weighing 150±10 g were obtained from NODCAR'S farm. The chosen animals were housed in plastic cages, every three females with one male. The animals were maintained on 14 hours artificial light and 10 hours complete darkness at normal atmospheric temperature (22±3^{o}C). All experimental animals were fed on standard diet and water *ad libitum*. . The animals were fasted before the sacrifice for about six hours.

The drugs used

a. Melatonin: It was a kind gift from Amoun Pharmaceutical Co., Egypt.

b. Primovlar: It was chosen as one of the widely used oral contraceptive tablets. The drug is a product of Chemical Industries Development (CID) under license of Schering.

Melatonin and primovlar were freshly suspended in 2% solution of Tween 80 in saline.

Experimental design

Vaginal smears were obtained daily by vaginal lavage. Two or more, 5 days regular estrous cycle in succession are required before the rats were accepted for experimentation. The selected animals were divided for two main experiments: -

Experiment 1

To investigate the effect of melatonin on the fertility of mature rats of both sexes, the following animal groups were chosen:

- Group I: Female rats (12 animals) treated with melatonin (1.0 mg/kg) 2 cycles before mating.
- Group II: Female rats (12 animals) treated with melatonin (1.0 mg/kg) on the first day of mating till 21 days.

- Group III: Male rats (12 animals) treated with melatonin (1.0 mg/kg) daily for two weeks before mating.
- Group IV: Control group (12 animals) treated with vehicle (2% solution of Tween 80 in saline).

Experiment II

To investigate the effect of primovlar on mature female rats, the following animal groups were chosen:

- Group I: Female rats (12 animals) treated with primovlar (1.0 mg/kg) 2 cycles before mating.
- Group II: Female rats (12 animals) treated with primovlar (1.0 mg/kg) on day one of mating till 21 days.

Rats in the treatment groups were given the defined dose of melatonin or primovlar (1.0 mg /kg) daily by gastric intubation.

The first detection of sperm in the vaginal smear was considered day one of pregnancy.

At the end of the experiments, semen was collected from males, and sperm count was done. Blood samples were obtained by sudden sacrifice of the animal and a part of the blood was collected on heparin at room temperature and the plasma was separated by centrifugation for determination of the circulating levels of FSH, LH, prolactin, estradiol and progesterone. Another part of the blood was left to coagulate at room temperature and then centrifuged at 3000 rpm and serum was collected for determination of total lipids and cholesterol. Slices of ovaries and testes were fixed for histological examination.

Methods

1. Vaginal smear

The vaginal smears were obtained daily at 9.00 am, using saline solution. The smears were allowed to dry and stained with ethylene blue. The methodology was according to Zarrow et al. (1964).

2. Semen examination

Semen collection was carried out either by electrical stimulation of ejaculatory centers (Zemjanis, 1970) or from seminal vesicles (Sarah and Maggie, 1998). The semen was diluted in physiological saline, and observation of spermatozoa was made in a low power (x 100) magnification of the microscope (Zemjanis, 1970).

Sperm count: Semen was diluted 1:20 in formalin solution (5g sodium bicarbonate, 1 ml 40% formalin and 100 ml distilled water) and the preparation was allowed to stand until the mucus dissolved. It was then shaken and a drop used to fill the counting chamber of a hemocytometer. Spermatozoa in a 1 mm^2 area (one large square) were counted and then multiplied by 200, 000 to obtain the number of spermatozoa per milliliter (John and Bauer, 1982).

3. Fertility index

The fertility index was determined adopting the method of Walter (1970). The number of fetuses that were alive on day 20 of pregnancy was divided by the number of swellings on day 11 of pregnancy.

4. Histological examination

For histological examination, ovaries and testes were removed, cleared from connective tissues and fat then fixed in 10% neutral buffered formalin, embedded in paraffin and sectioned. The sections were stained with hematoxylin and eosin (Carleton, 1976).

5. Hormone assays

The hormone assay was carried out using kits purchased from Diagnostic System Laboratories, Inc, Webster, Texas, USA.

Enzyme–Linked Immunosorbent Assay (ELISA) was used for determination of prolactin, FSH and LH in plasma. Prolactin was assayed according to the method of Liu et al. (1994). FSH and LH were determined according to Levine et al. (1985). Estradiol and progesterone in plasma samples were determined using immunoassay kit (EIA). The principle of the test is based on the competitive binding enzyme immunoassay format.

6. Biochemical analysis

Biochemical analysis was carried out using reagent kits purchased from Biodiagnostic, Egypt. Serum total lipids were analyzed according to the method of Zollner and Kirsch (1962) and serum cholesterol was determined according to the method of Richmond (1973) and Roeschlau et al. (1974).

7. Statistical analysis

The data were analyzed for the mean ± standard error (SE). Student's "*t*" test was conducted to find the significance (Campbell, 1989; Bailey, 1995).

Results

1. Vaginal smear changes in normal estrus cycle

At the beginning, irregularity and abnormality, if any, of the estrus cycle were studied. By and large the estrous cycle appeared to be regular. A complete cycle normally took about 4-5 days, which was divided into 4 phases, proestrus phase (10-20 hours), estrus phase (15-26 hours), metestrus phase (18-25 hours) and diestrus phase (55-70 hours), each with the respective characteristic cellular composition. The vaginal smear obtained during the first and the second day of pregnancy consisted of vaginal cells and condensed wavy sperm (Fig. 1a, b).

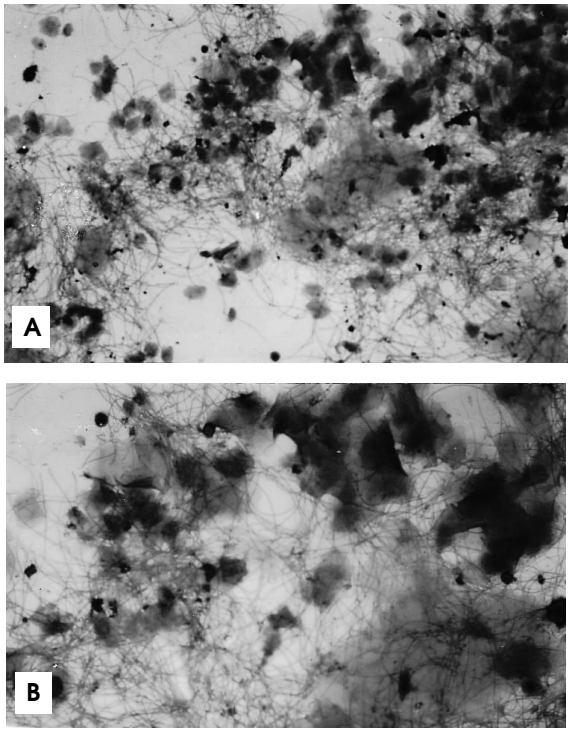

Fig. 1. Vaginal smear during pregnancy (methylene blue). A: Vaginal smear during the first day of pregnancy, showing cornified cells and condensed spermatozoa (x 50). B: Vaginal smear on the second day of pregnancy. Note wavy spermatozoa and vaginal cells (x 25) .

2. Histological changes

In the male rats treated with melatonin histological examination of the testes (Fig. 2b, c) revealed that the diameter of the seminiferous tubules was decreased when compared to the control (Fig. 2a). The majority of seminiferous tubules of the experimental animals appeared normal, with normal development of germ cells. Also, it was obviously clear that there was a marginal decrease in the abundance of Leydig cells.

In female rats treated with melatonin (Fig. 3b, c), histological examination revealed a slight loss of ovarian structure. The normal follicles came to be replaced with luteinized unrupted follicles similar to corpora lutea when compared with control animals (Fig. 3a). The female rats treated with melatonin for 2 cycles before mating showed numerous luteinized corpora lutea, and many of the granulosa cells forming the follicles possessed either pyknotic or hydropic degenerated nuclei. Follicular atresia was detected in the small immature follicles.

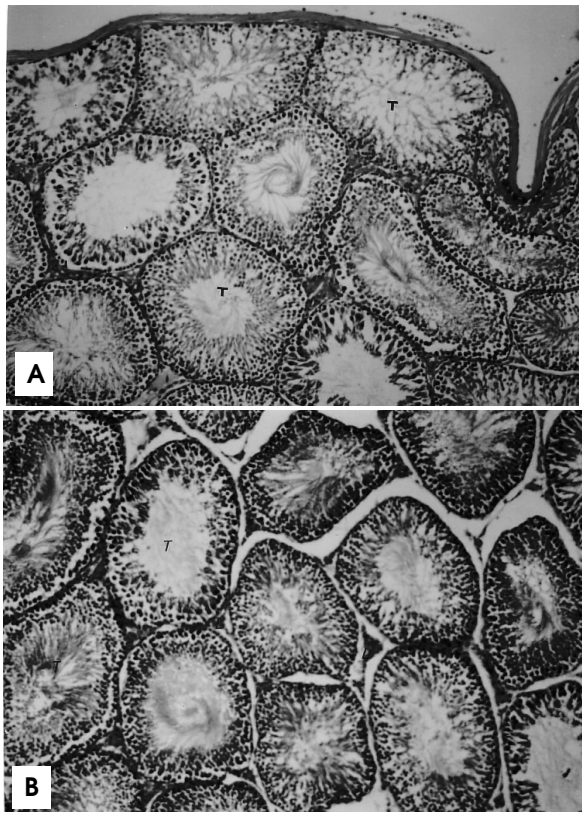

Fig. 2. A: Section of testis of a control rat showing normal seminiferous tubules (T) and normally distributed Leydig cells. B: Section of testis of a rat treated with 1.0 mg/kg of melatonin for 2 weeks before mating, showing decrease in size of seminiferous tubules and slight decrease in the abundance of Leydig cells (L). (H & E, x 150).

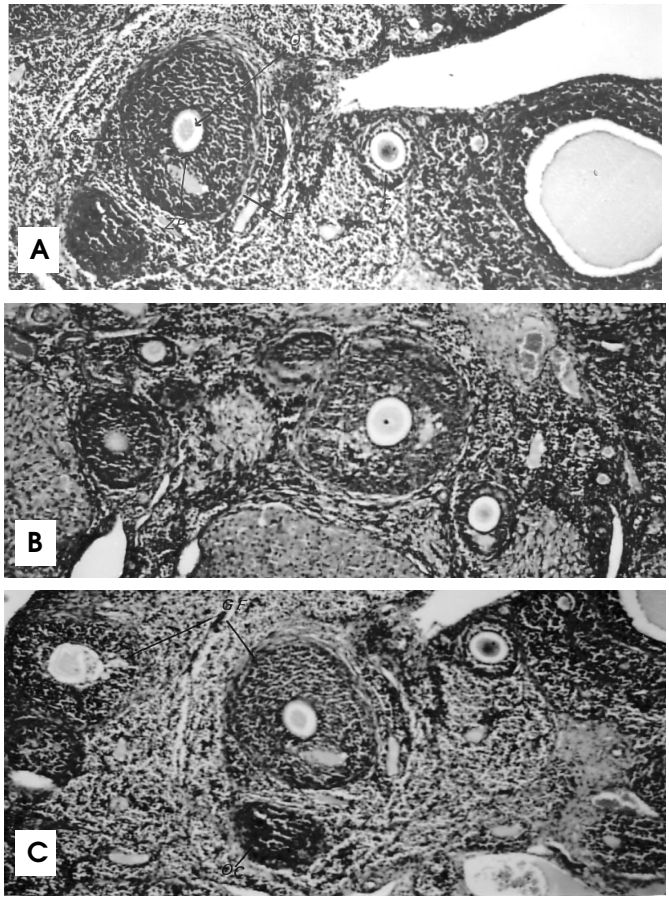

Fig. 3. A: Photimicrograph of ovary of control rat showing a multiluminar secondary follicle (F).The granulosa cells (G) have divided to produce a layer of cells. The pink–staining zona pellucida (ZP) is apparent between the oocyte (O) and the granulosa cells. B: Photomicrograph of ovary of rat treated with 1.0 mg/kg of melatonin for 2 cycles before mating, showing Graffian follicles at many stages surrounded with numerous corpora lutea, luteinized unruptured follicles, pyknosis and vacuolization of granulosa cells. C: Photomicrograph of ovary of rat treated with 1.0 mg/kg of melatonin from day 1of mating till 21 days, showing ovarian cyst (OC). (H & E, x 250).

3. Pregnancy percentage

The data concerning the effect of melatonin or primovlar on the pregnancy percentage (Fig. 4) revealed a marked decrease in all studied female groups. The female rats treated with melatonin for 2 cycles before mating had the pregnancy stopped completely. Also, in the case of normal females mated with males treated with melatonin two weeks before mating, the percent pregnancy decreased, as compared to control.

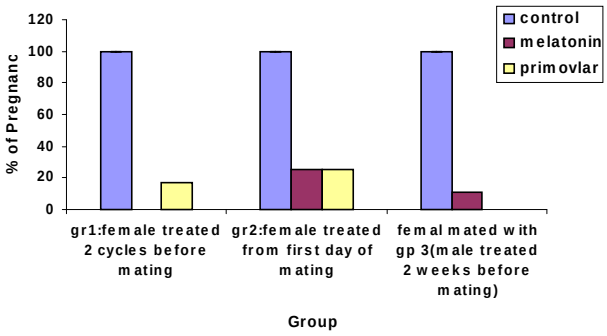

Fig. 4. Percentage of pregnancy in female rats after administration of 1.0 mg/kg of melatonin or primovlar. Asterisk (*) denotes significant change from control.

4. Fertility (Pregnancy) index

Data presented in figure 5 show significant decrease ($p < 0.01$) in fertility index of all the groups studied, and fertility dropped to zero in female rats treated with melatonin two cycles before mating.

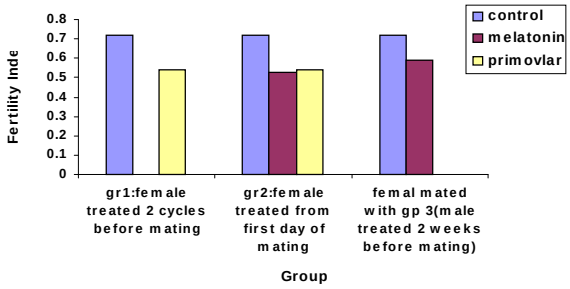

Fig. 5 : Fertility index of female rats after administration of 1.0 mg/kg of melatonin or primovlar. Asterisk (*) denotes significant change from control.

5. Sperm count and motility

The data on the sperm count (Fig. 6)) reveal non-significant effect of the tested dose of melatonin. As regards the sperm motility, the waves of the sperm were not observed and the sperm became immotile and engaged in poor pendular motility. Also, the tested dose of melatonin resulted in death of sperm 60 min after collection, and the dead sperm increased to 80% where as in control rats it was only 20%. The sperm showed different forms of abnormalities.

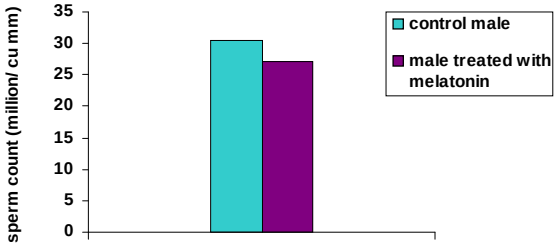

Fig. 6. Effect of melatonin at dose 1.0 mg/kg on the sperm count of male albino rats.

6. Plasma hormone levels

The effect of tested drugs on plasma hormone levels is represented in table 1.

a) Prolactin

Data recorded for the effect of melatonin or primovlar on prolactin level revealed significant increase ($p<0.01$) in all groups studied. Melatonin administration induced more potent effect than primovlar. The most potent effect was recorded in female group treated 2 cycles before mating. In the male rats treated with

Table 1: Hormone levels of adult male and female rats after treatment with 1.0 mg/kg of melatonin or primovlar.

Hormones	Control (female)	Group I (Female treated 2 cycles before mating)		Group II (Female treated from the first day of mating till 21 days)		Group III (Male treated 2 weeks before mating)	
		Primovlar	Melatonin	Primovlar	Melatonin	Control	Melatonin
Prolactin (ng/ml)	4.53 ± 0.48	34.19 ± 2.43*	97.5 ± 4.83*	16.11 ± 1.30*	54.60 ± 2.90*	5.84 ± 0.65	30.08 ± 1.76*
FSH (mIu/ml)	1.82 ± 0.06	1.12 ± 0.05*	0.95 ± 0.07*	1.11 ± 0.05*	0.82 ± 0.08*	1.85 ± 0.04	1.05 ± 0.06*
LH (mIu/ml	1.76 ± 0.04	0.80 ± 0.08*	0.75 ± 0.09*	0.68 ± 0.10*	0.50 ± 0.07*	3.02 ± 0.15	0.73 ± 0.09*
Estradiol (pg/ml)	154.16 ± 7.36	39.71 ± 3.52*	31.02 ± 2.56*	30.33 ± 3.09*	25.78 ± 2.44*	166.31 ± 7.56	32.90 ± 2.60*
Progesterone (ng/ml)	47.78 ± 2.27	4.95 ± 0.62*	4.32 ± 0.39*	5.16 ± 0.52*	3.16 ± 0.62*	40.72 ± 1.44	3.07 ± 0.36*

Data are expressed as Mean ± S.E. Number of animals in each experiment was 12. Statistical analysis was done based on Student's "*t*" test: * $P<0.05$ significant.

Table 2: Effect of melatonin or primovlar at dose 1.0 mg/kg on serum total lipids and cholesterol levels of male and female rats.

Parameter	Control (Female)	Group I (Female treated 2 cycles before mating)		Group II (Female treated from the first day of mating till 21 days)		Group III (Male treated 2 weeks before mating)	
		Primovlar	Melatonin	Primovlar	Melatonin	Control	Melatonin
Total lipid (mg/dl)	208.67±4.18	401.9±23.4*	523.58±28.8*	447.00±32.41*	489.77±38.98*	202.83±4.79	495.41±18.6*
Cholesterol (mg/dl)	92.67±4.92	153.30±6.64*	153.92±6.19*	142.44±4.93*	151.88±5.71*	94.25±4.83	143.00±6.56*

Data are expressed as Mean ± S.E. Number of animals in each experiment was 12. Statistical analysis was done based on Student's "*t*" test: * $P<0.05$ significant.

melatonin for two weeks before mating, a significant elevation ($p<0.01$) in prolactin level was recorded.

b) Follicular stimulating hormone (FSH) and luteinizing hormone (LH)

The results revealed significant decrease ($p<0.01$) in FSH and LH levels in all treated groups. The hormone levels of female rats treated with melatonin were the most affected when compared with those treated with primovlar. The decrease in FSH level was highly pronounced in female rats treated for 2 cycles before mating, while the reduction in LH level was highly pronounced in females treated from day one of mating. As regards to the male rats treated with melatonin, the recorded levels of FSH and LH decreased significantly ($p<0.01$).

c) Estradiol and progesterone

The data recorded revealed that daily administration of melatonin or primovlar to female rats caused significant decrease ($p<0.01$) in estradiol and progesterone levels. This decrease was more pronounced in females treated with melatonin from day one of mating. Also, administration of melatonin to male rats for two weeks before mating caused significant decrease ($p<0.01$) in estradiol and progesterone levels.

7. Total lipids and cholesterol

Changes in total lipids and cholesterol due to the treatments are presented in table 2.

From the data it is evident that total lipids and cholesterol increased to significant levels ($p<0.01$) in all treated groups and the increase was more marked in animals treated with melatonin than with primovlar. On the other hand, treatment with melatonin 2 cycles before mating produced more effect in case of total lipids while in the case of cholesterol, there was no difference in the increase between rats treated for 2 cycles before mating and those treated from the day one of mating.

Discussion

Melatonin is an evolutionarily highly conserved molecule that plays an important role in transduction of the clock and calendar information to all living organisms including man. The mode of action and multiplicity of melatonin effects in human and other animals is complex, and in some cases obscure. The present study aimed at investigating the effect of the tested dose of melatonin on the fertility of male and female albino rats to clarify its mode of action.

The effect of the tested drugs (melatonin or primovlar) on the pregnancy percentage of female rats revealed a marked inhibitory effect in all the groups. Our results are in agreement with the findings of Arendt (1996), and can be explained on the basis of melatonin action. Melatonin acts via specific membrane receptors which have been demonstrated in several brain areas with high concentrations in the hypothalamic suprachiasmatic nucleus and the pars tuberalis of the pituitary (Juszczak and Michalska, 2006a, b). It was reported that the hypothalamus-pituitary axis plays a critical role in the regulation of reproduction by melatonin (Schuster, 2007; Prendergast, 2010). In addition, melatonin binding sites or melatonin receptors have been identified in the testis, epididymis, vas deferens, prostate, ovary and mammary gland (Drew et al., 2001). Melatonin can, therefore, influence gonadal function indirectly via its effect on gonadotropin-releasing hormone and /or gonadotropin secretion. It may also act directly on the reproductive system (Boczek-Leszczyk and Juszczak, 2007). These concepts will interpret the decrease in the percentage of pregnancy as a result of melatonin administration.

Comparably with the reference drug primovlar, an oral contraceptive, the percent of pregnancy was increased in rats treated with primovlar 2 cycles before mating as compared to melatonin-treated group. So, melatonin has been investigated as contraceptive when given at higher doses (Arendt, 1996; Suhner and Steffen, 1997).

Melatonin treatment leads to decline in the fertility index due to the resorption of some implanted fertilized ova. The resorption resulting from melatonin administration leads to reduction in the number of implanted fertilized ova, which may stimulate gonadal cell division and lead finally to increase in gonad weight. The present findings agree with the result of Hamed et al. (1991) who found that melatonin administration to mice on the 4^{th} day of pregnancy leads to interruption of pregnancy and reduction in the mean number of implanted sites, pointing to use of melatonin as post-coital contraceptive agent.

The present study also revealed that melatonin administration caused non-significant variation in the sperm count. Melatonin affected sperm motility and led to death of sperm collected 60 minutes after administration. The sperm also showed abnormality in its forms. These changes resulted in decrease of potency of male rats and may be the cause for decrease of percent pregnancy in female rats mated with the treated males. These observations are in agreement with Gwayi and Bernard (2002) who reported that melatonin had negative effects on forward progression of sperm and the quality of sperm motility in rats. Also, Tanyildizi et al. (2006) showed that melatonin to bull caused decrease in semen decreased of spermatozoal motility in a time- and dose-dependant manner. Studies of Yie et al. (1991) and Luboshitzky et al. (2002) revealed direct inhibitory effect of melatonin on testicular and epididymal aromatase activity resulting in an altered androgen/estrogen milieu and, consequently, decreased sperm count and motility. Malpaux et al. (1999) found a negative reletionship between sperm production and melatonin secretion in male rats. The study reported that the nocturnal secretion of melatonin regulates the pulsatile release of GnRH from hypothalamus. Change in GnRH release in turn affects luteinizing hormone secretion and leads to decrease of sperm production. The study further revealed that melatonin does not seem to act directly on GnRH neurons, rather it appears to involve a complex neural circuit of interneurons that include at least dopaminergic, serotoninergic and excitatory amino acidergic neurons. Sirotkin & Schaeffer (1997) suggested that melatonin exerts its antigonadal effects, at least in part, through the direct decrease of testosterone production.

In the present study, melatonin treatment led to significant increase in the circulating level of prolactin in male as well as female rats. The treatment of female rats with melatonin two cycles before mating exerted a more effect in prolactin level than in the treatment from the first day of mating. Concurring with the present study, Brzezinski et al. (1994) stated that plasma prolactin concentration in rats and human rise in response to melatonin administration. Prolactin secretion is regulated by complex mechanisms involving hypothalamic secretion of dopamine. Any process interfering with dopamine synthesis, its transport to the pituitary gland, or its action at the level of lactotroph dopamine receptors can cause hyperprolactinemia (Mancin et al., 2008). Melatonin significantly reduces the inhibitory effect of dopamine on prolactin secretion, and so prolactin levels were increased due to melatonin administration (Rozell and Mead, 1993). The significant rise in prolactin level may be correlated with progesterone levels as reported by Kaplan et al. (1991). In the present study, melatonin treatment caused decrease in progesterone level and this may lead to elevation of prolactin. In addition, melatonin may also lead to decrease in uterine prolactin receptors as found by Rose et al. (1996) and this might cause elevation in plasma prolactin level. Terzolo et al. (1991) suggested that melatonin may play a facilitory role in the thyrotropin releasing hormone (TRH) action leading to elevation of prolactin level. According to Juszczak et al. (2006), melatonin responsive pars tuberalis of the pituitary is an intermediary in the control of prolactin secretion. Johnston (2004) reported that melatonin may affect the female hormones and thereby help to regulate menstrual cycle via its relationship with prolactin. The melatonin surge is followed by a surge in prolactin. The pathological state of hyperprolactinemia is associated with suppressed gonadal function. Prolactin appears to decrease the pulsatile secretion of FSH and LH without affecting the basal levels of these hormones. Prolactin also suppresses the mid-cycle surge of LH. This suppression would effectively inhibit ovulation.

The present study showed significant decrease in the circulating levels of follicle stimulating hormone (FSH) and luteinizing hormone (LH) in female and male rats treated with melatonin. The finding is in agreement with the reported of Fernandez et al. (2000) that gonadotropins and prolactin release from hemipitutaries of young rats was decreased by melatonin treatment. Bellipanni et al. (2001) reported that in a controlled trial, 3 mg of melatonin at bedtime evidently suppressed LH in young women as compared to placebo. According to Hattori et al. (1995), melatonin can act directly on the pituitary gland to suppress LHRH-induced release of LH by a mechanism which eventually involves down-regulation of melatonin receptors. These findings are also supported by the studies of Wojtowicz and Jakiel (2002)

and Kripke et al. (2006) who revealed that melatonin acts negatively on hypothalamus and inhibits GnRH release and in turn decreases LH and FSH secretion. This view was also supported by Lawson et al. (1992) who reported reduction of serum levels of FSH and LH by causing 50-70% decrease in the number of estrogen receptors in medial preoptic area neurons and thus influencing steroid feed back mechanisms post-melatonin administration. This view was supported by Vanecek (1999), who stated that melatonin inhibits Gn-RH release of LH and FSH by inhibiting Gn-RH induced Ca^{++} release from the endoplasmic reticulum as well as Ca^{++} influx through voltage sensitive channels. Melatonin also inhibits Gn-RH induced accumulation of cAMP which may result in the decreased influx of Ca^{++}, because cAMP, through protein kinase A, stimulates Ca^{++} influx into the gonadotrophs (Schuster, 2007).

The data of the present study also showed significant decrease in the level of both estrogen and progesterone in male as well as female rats as a result of melatonin treatment. Many research workers have reported similar findings for both animals and human. Voodrouw et al. (1992) stated that daily administration of 300 mg melatonin for a period of 4 months caused significant decrease in the level of progesterone and estrogen in women compared to non-medicated control groups. The study of Tamura et al. (1998) revealed that melatonin treatment decreased estradiol and progesterone production by the preovulatory follicles in a dose- and duration-dependant manner. Pawlikowski et al. (2002) recorded significant decrease in estradiol concentration after 6 months of melatonin treatment at a dose 2 mg daily in elderly women. In another study, Okatani et al. (1997) found that estrogen but not progesterone might be the ovarian hormone responsible for the inhibition of pineal melatonin synthesis observed in the normal cycling female rats. This inhibitory effect of estrogen on melatonin synthesis appeared to be mediated by a change in norepinephrine-induced stimulation of pineal adenylate cyclase activity. Also, San Martin and Touritou (2000) reported a direct effect of progesterone on pineal melatonin release and strongly suggested a time–related effect of progesterone on pineal function. Abd-Allah et al. (2003) reported that in non-pregnant rats treated with melatonin at a dose of 0.8mg/kg per day for 15 consecutive days, a significant reduction in the number of uterine estrogen receptors with concomitant increase in progesterone receptors was recorded as compared to control ones.

Recent studies conclude that melatonin interacts with estrogen-signaling pathways through theree different mechanisms :(a) direct neuroendocrine mechanisms that include melatonin down-regulation of the hypothalamic-pituitary-reproductive axis and the consequent reduction of circulating level of estrogen, (b) direct melatonin action by interacting with the activation of estrogen receptors, thus behaving as selective estrogen receptor mediator, and (c) the regulation of the enzymes involved in biosynthesis of estrogen in peripheral tissues, thus behaving as selective estrogen enzyme modulator. As melatonin reduces the activity and expression of aromatase (the main enzyme responsible for local synthesis of estrogen), sulfatase (the enzyme that catalyzes the rate-limiting step in the conversion of estrogen sulfates to estrogen) and 17-beta-hydroxysteroid dehydrogenase (the enzyme which converts the relatively inactive estrone to the most potent 17-beta-estradiol) and increase the activity and expression of estrogen sulfotransferase, it may protect mammary tissues from excessive estrogenic effect (Cos et al., 2008; Gonzalez et al., 2007, 2010). The decrease observed in circulating levels of estrogen and progesterone may also be explained on the basis of lowering levels of LH and FSH, which was recorded in the present study also. The release of both pituitary FSH and LH is controlled by feed-back mechanisms involving circulating levels of estrogen and progesterone as reported by McNeilly et al. (2003) and Zhao et al. (2009). It is known that after ovulation occurs through rupture of follicle, the follicle is transformed into a corpus luteum, and progesterone levels are clinically useful to confirm ovulation. So, in the present study, the inhibition of ovulation induced by melatonin treatment led to significant decrease in progesterone level.

The present study also investigated the effect of tested drugs on serum total lipid and cholesterol. Recent studies have suggested that lipids and fatty acids composition may be important determination of fertility .The data of the current study concerning the plasma total lipid showed significant elevation in male and female rats

all over the study stages. The elevation in serum total lipid may be due to the decrease in estrogen concentration, as a result of melatonin treatment, and this in turn cause both poor distribution of fat and defect disposition of subcutaneous adipose tissue in mature rat (Robert et al., 1996). The increase in serum total lipid may be reflecting the significant decrease in lipid peroxidation which occurred after chronic melatonin administration (Hoyos et al., 2000). Also, Mori et al. (1989) explained that melatonin diminished the fatty infiltration in the liver of animals and this in turn leads to elevation of lipid level in blood.

The recorded data for the effect of melatonin administration on serum cholesterol level revealed significant increase in cholesterol in male and female rats. Our results are in agreement with that of Darul and Kruczynska (2004) who recorded a significant increase in the level of total cholesterol and triglycerides as a result of treatment of dairy cow with 0.1 mg /kg body weight of melatonin. Also, Fabis et al. (2002) reported that melatonin augmented significantly the concentration of total, free and esterified cholesterol as well as high density lipoprotein cholesterol in the blood of male Wister rats. This elevation in cholesterol level may be caused by the reduction in estrogen and progesterone levels due to melatonin treatment. This explanation depends on the scientific rule that cholesterol pool is the main precursor of the ovarian hormones (Robert et al., 1996; Kamada et al., 1997) thus the depletion in ovarian hormonal level lead to elevation in cholesterol.

In conclusion, the present investigation demonstrated that the tested dose of melatonin had an inhibitory effect on the fertility of both male and female rats. This effect may be attained through the previously different suggested mechanisms.

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