



Restoring Action of affected Prostate Gland Disorder by Hyperprolactinemia through Melatonin Treatment

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Abstract

Hyperprolactinemia is primarily an endocrine disorder in men. It can be caused by a variety of factors, including medication use, hypothyroidism, and pituitary disorders. Melatonin is known as an antioxidant; moreover, it has multiple uses in biological rhythm regulation. Our study aims to investigate the effects of melatonin supplements on prostate gland protection in metoclopramide-induced hyperprolactinemic rats. A total of twenty-four male rats were divided into four groups: The first group received only normal saline. The second group received metoclopramide 5 mg/kg orally to induce hyperprolactinemic rats. The third group received metoclopramide-induced hyperprolactinemic rats plus bromocriptine 2.5 mg/kg. The fourth group received metoclopramide-induced hyperprolactinemic rats administered melatonin at 2.5 mg/kg. After treatments, hormonal parameters (prolactin, testosterone, FSH, and LH) were analyzed, and the prostate gland was collected as well as routine paraffin-embedded section staining with hematoxylin and eosin. The result of the study revealed a significant decrease ($P \leq 0.05$) in prolactin levels and a significant increase ($P \leq 0.01$) in testosterone, FSH, and LH levels in Group 3 and Group 4 compared with Group 2. In addition to histological changes after treatment with MT, all prostate components displayed structural tissue that was nearly normal. The glandular epithelium's height was reduced, the follicular lumen was expanded, and secretion materials were homogeneously distributed. Also, note the vanishing of intraluminal invagination and the presence of normal stroma in a regular shape. The study concluded that melatonin had an encouraging effect on the prostate via the stabilization of sex hormones and the reconfiguration of histostructures.

1803

Key Words: Hyperprolactinemia, Prostate Gland, Prolactin (PRL), Melatonin (MT), (BRC).

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Introduction

Hyperprolactinaemia is a relatively common endocrine abnormality characterized by a high prolactin concentration in the blood. The causes range from minor medical issues that do not require treatment to major medical issues that require immediate treatment (Chen & Burt, 2017). It is the most common hypothalamic-pituitary axis disorder (Akter et al., 2018). Hyperprolactinemia can be caused by a variety of physiological, analytical, pathological, and pharmacological factors (Bernard et al., 2015; Vilar et al., 2019).

Prolactin excess in men can be associated with gynecomastia (rarely galactorrhea), sexual dysfunction, and infertility (Rizzo et al., 2020). It can also cause decreased libido, osteopenia or osteoporosis, and an increased risk of fracture in both sexes (Melmed et al., 2011; Capozzi et al., 2015).

Melatonin (MT) is a tryptophan derivative that is produced by pinealocytes as well as other cells in vertebrates (Gonzalez Arto et al., 2016; Reiter et al., 2020).

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Melatonin has the ability to regulate a wide variety of physiological activities, including the well-known sleep-wake cycle and circadian rhythm modulation, as well as neural development, immune system preservation and regulation, and endocrine functions (Amaral & Cipolla-Neto, 2018). Bromocriptine (BRC) is a semi-synthetic derivative of ergocryptine that stimulates dopaminergic receptors (D2) in the pituitary gland. It is a substance that has a wide range of effects on organisms. It inhibits the production and secretion of prolactin from the pituitary gland's anterior lobe (Moloth, 2015).

Materials and Methods

Experimental Animals

The twenty-four adult male Albino rats were provided by the Iraqi Center for Cancer Research and Medical Genetics/Ministry of Higher Education and Scientific Research. These rats were between the ages of 3 and 4 months, and their weights ranged from 190 to 240 g/bw). The animals were housed in the Animal House of Thi-Qar University College of Education for Pure Science, and the room was under conventional conditions of temperature ($24\pm 1^{\circ}\text{C}$) under a 12 hour light/dark cycle. Before the experiment began, the rats were given 10 days to acclimate and were given free access to food and water.

Experimental Design

Following the acclimatization period, the animals were divided into four groups of six rats each. The first group (the control group) was given 0.5 mL of distilled water only. The second group was given metoclopramide 5 mg/kg b.wt orally using gavage for 21 days to induce hyperprolactinemia according to Uhuo et al., 2021. The third group was given BRC at 2.5 mg/kg b.wt intraperitoneally (Uhuo et al., 2021). The fourth group was given MT 2.5 mg intraperitoneally (Koohsari et al., 2020).

Biochemical Analysis

Determination of Serum Prolactin, Testosterone, FSH, LH, Levels

Prolactin (PRL), testosterone, follicle-stimulating hormone (FSH) and luteinizing hormone (LH) were determined using commercial kits (Vidas, bioMérieux, France).

Histopathological Examination

The prostate gland was fixed in formalin solution (10%) for 48 hours. They were then treated (washed with water, passed through ascending grades of alcohol, cleared in xylene, and embedded in paraffin wax at 70°C). $5\mu\text{m}$ of tissue thickness was mounted on clean glass slides and stained with hematoxylin and eosin according to Bancroft and Gamble (2008).

Statistical Analysis

All data is expressed as the mean \pm standard deviation. One-way analysis of variance was used to compare the differences between groups, while multiple comparisons were performed by Tukey's test as a *post-hoc* test. ($P\leq 0.01$) was considered statistically significant.

Results and Discussion

The results presented in Figure (1) indicate a significant decrease ($P\leq 0.01$) in the level of PRL hormone in the G1 compared with the G2 and a non-significant decrease compared with the G3 and G4. As for the G2, it showed a significant increase compared with the G3 and the G4. PRL levels decreased gradually in G3 and G4. As for the G3, it showed a non-significant increase with the G4. The results shown in Figure (2) indicate a significant decrease ($P\leq 0.01$) in the level of testosterone in G1 compared to G2, G3 and G4. The G2 show significantly declined compared with G3 and the G4. Also G3 offer non significantly decreased with G4.

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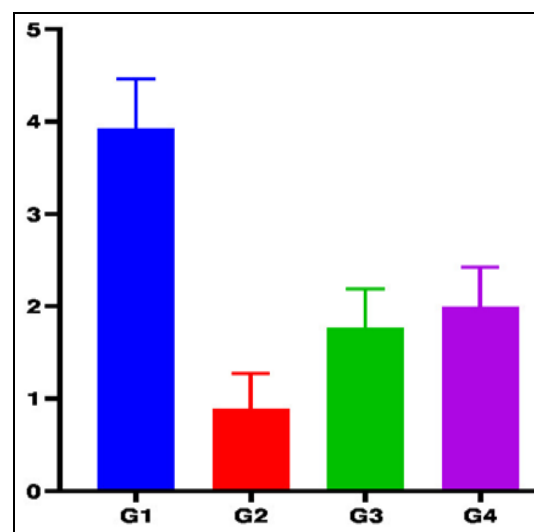


Figure 1. PRL hormone level in all groups

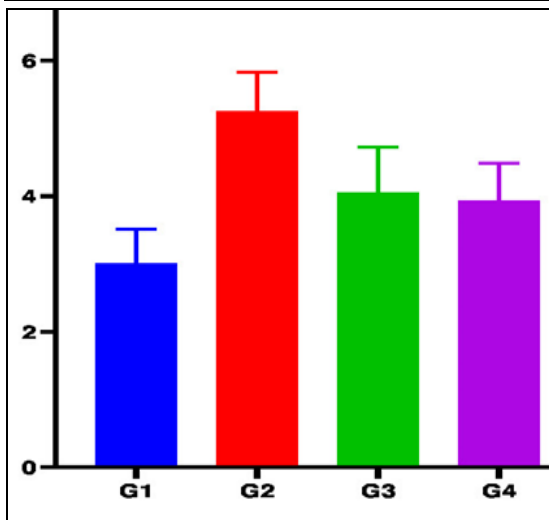


Figure 2. Testosterone hormone level in all group

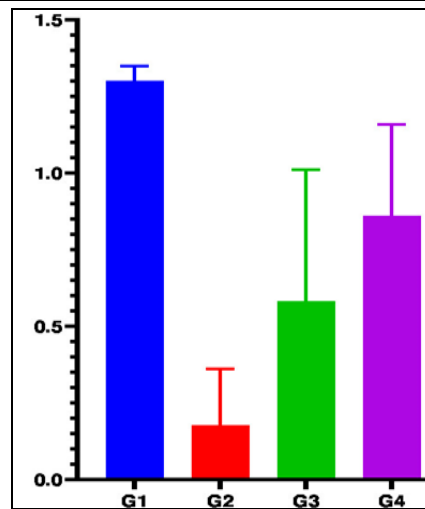


Figure 4. LH hormone level in all groups

As for the hormone FSH the results in Figure (3) indicated that in the G1 there was a significant increase ($P \leq 0.01$) with the G2 and with G3 and non-significant increase with G4. Regarding the G2, there was significant decline with the G3, and G4. The G3 did not display a significant decrease with the G4. The results of the LH hormone in Figure (4) showed a significant increase ($P \leq 0.01$) in the G1 compared other groups. As for the G2, it showed a significant decline with the G3 and G4. Regarding the G3, it did not show any significant effect with the G4.

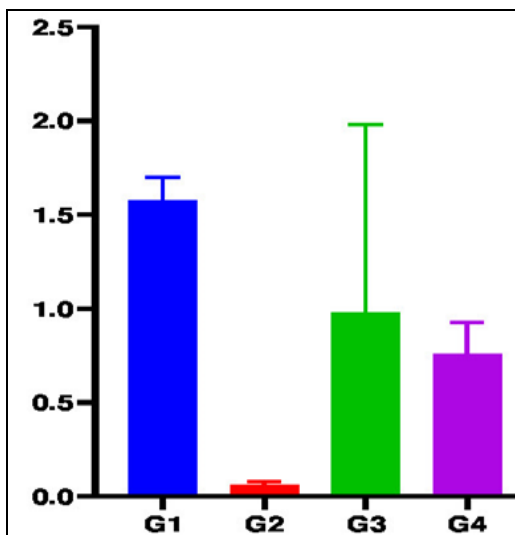
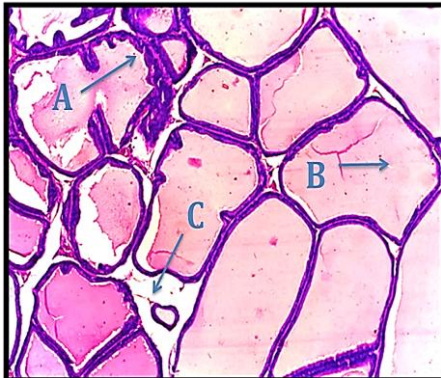
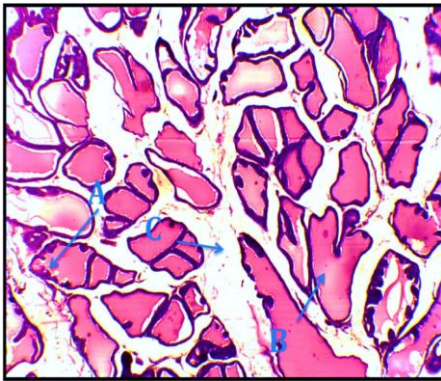


Figure 3. FSH hormone level in all groups

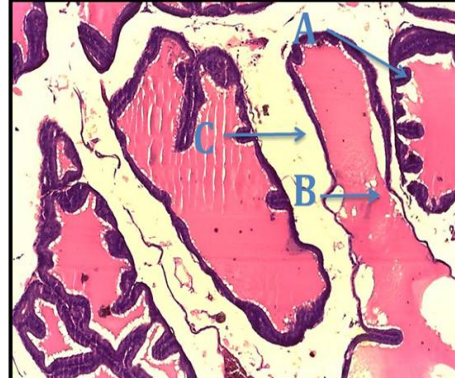
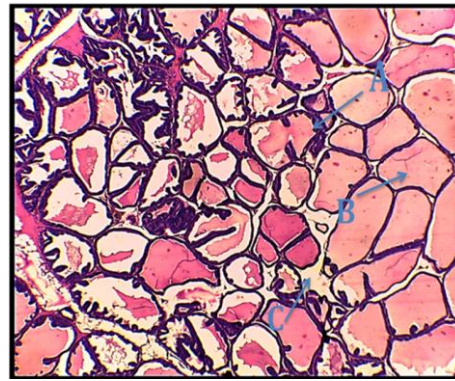
Histological Changes

In control rats, histological results revealed that prostate structures had normal architectural organization that was apparent with the glandular epithelium encircling the follicular lumen of the prostate. Furthermore, homogenetic serous secretions act as colloid materials. The epithelium of acinal is made up of columnar cells that repose on the basement membrane, with basal cells disseminated between them. In between the tubules, scattered stroma diffuses into intra interstitial space. In the metoclopramide-induced hyperprolactinemia rats group, prostatic epithelial cell hyperplasia was observed, as well as increased intraluminal budding, giving the metaplasia figure, but columnar cells transforming into cuboidal or flattened shapes. Calcificated and non-homogeneous secretion materials are found in the follicular lumen. In some prostatic follicles, this material has vanished. In addition to prostatic Follicles develop an irregular shape and undergo hypertrophy. A sparse stroma was missing, resulting in adherent follicles. The components of the prostate in the rat group treated with BRC begin to improve and respond to treatment more positively and better than in the previous group, and the secretory substance begins to restore its activity, but intraluminal immersion is present. According to the findings, all of the rats in the MT-treated group had prostate constituents that were repaired and emerged with nearly normal structural tissue. The glandular epithelium height was decreased, and the lumen of the follicular was magnified, with dark secretion materials being found characterized by homogeneity, as well as the disappearance of intraluminal invagination and the appearance of normal stroma in regular form.

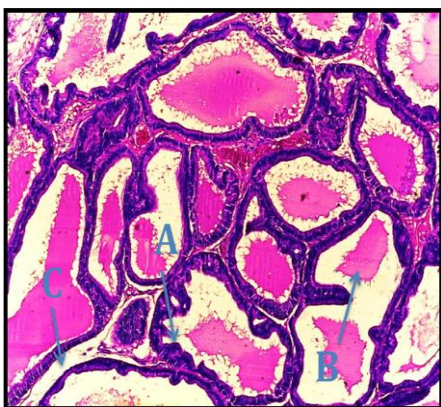
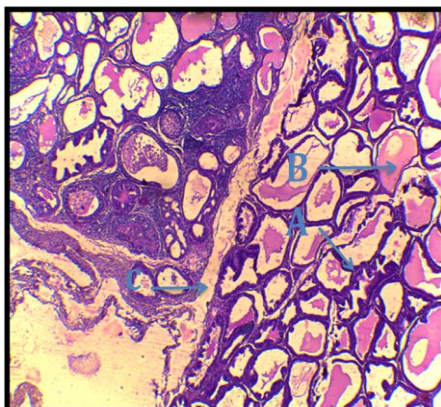




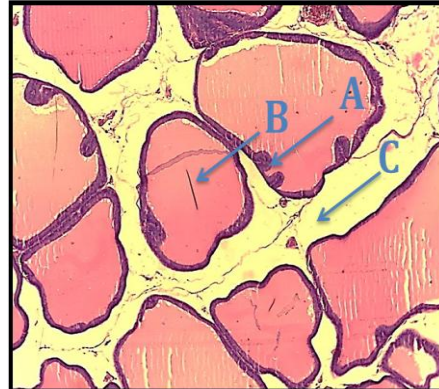
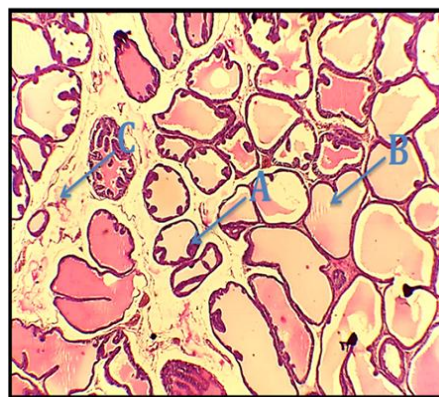
A. Section of normal histostructures of prostate tissues in control group. Columnar cells of glandular epithelium (A), secretion material (B), interstitial stroma (C). Photomicrograph X (40) And X (100) (left and right) respectively



C. Section of prostatic histostructures in BRC- treated group. Columnar cells of glandular epithelium (A), secretion material (B), interstitial stroma (C). Photomicrograph X (40). And X (100) (left and right) respectively.



B. Section of prostatic histostructures in metoclopramide treated group. Columnar cells of glandular epithelium (A), secretion material (B), interstitial stroma (C). Photomicrograph X (40). And X (100) (left and right) respectively.



D. Section of prostatic histostructures in MT- treated group. Columnar cells of glandular epithelium (A), secretion material (B), interstitial stroma (C). Photomicrograph X (40). And X (100) (left and right) respectively.

Discussion

This study investigated the efficacy of melatonin in lowering prolactin levels in male rats with hyperprolactinemia. Our results indicated that the prolactin levels were decreased in both the BRC and melatonin groups after four weeks of treatment. Nevertheless, both MT and BRC are equally effective in reducing prolactin levels. But MT has an effective role in improving tissue and returning it to its normal state.

Hyperprolactinemia is one of the most common pituitary clinical disorders is caused by a prolactinoma or another disorder in the hypophyseal system. Disorders in gonadotrophic hormone secretion are caused by excessive prolactin secretion. As a result, the male libido declines or disappears, causing depression (Molik & Blasiak, 2015). In the current study, we observed a significant increase in PRL levels and a decrease in testosterone, FSH and LH levels in male rats after administering metoclopramide. It is consistent with previous findings (Molitch, 2005; Coker and Taylor, 2010; Majumda and Mangal, 2013; Almasseri et al., 2020; Uhuo et al., 2021). In addition, rats in Group 2 with hyperprolactinemia had lower testosterone, FSH, and LH levels. Several hypotheses have been proposed to explain the mechanisms of testosterone deficiency in hyperprolactinemia animals. PRL has receptors found in all parts of the body, especially in the reproductive system. These receptors are linked with prolactin and perform its function. As shown by Khouardaji et al. (2018), PRL receptor expression has been found in Leydig cells, Sertoli cells, and germ cells. The reason could also be due to PRL's effect on the brain and the hypothalamic-pituitary axis, which negatively affects the secreted hormones. Tsutsumi & Webster (2009) and Bernard et al. (2015) suggest that hyperprolactinemia reduces hypothalamic kisspeptin production, reducing hypothalamic secretion of GnRH (gonadotrophin-releasing hormone) and, as a result, the decline of pituitary gonadotrophin synthesis and secretion (LH and FSH), resulting in gonadal stimulation loss and infertility.

PRL can influence steroidogenesis by controlling the activity of steroidogenic enzymes or through modulating LH receptor expression (Binart et al., 2003; Rastrelli et al., 2015). Therefore, a decrease in testosterone levels in the hyperprolactinemia group was confirmed in our study through indugious histological changes in the prostate tissue structures, which indicated aberrant

histological changes. The findings suggest that hyperprolactinemia produces morphological abnormalities in the dorsal lobe of the prostate in male rats, according to the findings. Elevated prolactin, either directly or indirectly through a reduction in testosterone, causes the problems (Suczanowska-Gabowska et al., 2006). In our results for the current study, a decrease in PRL and an increase in testosterone, FSH, and LH levels were found in groups of rats treated with BRC. It lowers (PRL) concentrations in hyperprolactinemia (Molitch, 2015). Also, according to research (Dominguez-Gonzales and Genaro, 1994), dopaminergic receptor stimulation in the hypothalamo-pituitary area suggests a considerable role for dopamine in regulating the release of sheep (PRL).

As mentioned by Andrews et al. (2001), in BRC-treated ewes, the concentration of PRL in blood plasma reached less than 1.5 ng/ml, which may be sufficient to inhibit tuberoinfundibular neurons. Our results do not correspond with those of Besognet et al. (1995), whose statistics show no significant changes in PRL concentration after BRC was administered to animals. This result corresponds with Boucher et al. (1977), who investigated the effects of BRC in hyperprolactinemia patients and found that prolactin secretion was reduced. The results of the current study indicate that the MT-treated group showed a decrease in PRL levels and an increase in testosterone, FSH, and LH levels. As it's known, MT regulates a variety of biological pathways, including hormone secretion and modulation, circadian rhythms, and reproduction (Kim et al., 2012; Maroufi et al., 2020). According to Villana et al. (1989), MT appears to modify prolactin secretion via multiple complex mechanisms that may be dependent on the animals' physiological status (hormonal and neurotransmitter). The decrease in PRL levels may be MT and may inhibit prolactin secretion in the pituitary gland. According to research (Ciechanowska et al., 2013), long-term administration of MT to both rams and ewes (e.g., using subcutaneous MT implants during the long day) inhibited PRL secretion. Also, Morgan (2000) mentioned that the long MT signal from the short photoperiod or a constant signal produced by the implantation of MT may inhibit PRL secretion by reducing the release of tuberalin from the pars tuberalis.

Or it may be due to the effect of melatonin on the activity of dopamine in the brain and the regulation



of vital processes. As reported by Molik et al. (2013). There was a decrease in dopaminergic system activity and a decrease in PRL concentration as a result of the action of exogenous MT. This result does not correspond with Terzolo et al. (1991), who showed that PRL was unaffected by MT action in one of their experiments. The increase in testosterone levels in the current study may be due to the difference in testosterone concentrations could be due to the relationship between melatonin, photoperiod, and GnRH, where MT administration influenced the secretion of LH and GnRH (Valenti et al. 1997, Valenti et al. 1999). Or, melatonin treatment may stimulate hypothalamic cells to release GnRH, which affects gonadal function, or it may increase GnRH RNA in the hypothalamus (Bernard et al., 1999). The increase in testosterone level is consistent with melatonin's protection of Leydig cells because of the presence of MT binding sites in the reproductive system (Ghasemi, 2010). Also, several studies in rodents have shown that melatonin activates the hypothalamohypophyseal-gonadal axis and, as a consequence, acts as a modulator of testosterone release (Reiter et al., 2013). This study corresponds with Majrashi et al. (2017) and not with Mahmud et al. (2013), which show MT administration significantly decreases both LH and testosterone levels, and MT prevents testosterone secretion via the hypothalamo-pituitary axis. Moreover, the pineal gland and the testis have a functional relationship and feedback regulation (Yilmaz et al., 2000). As for the histological changes, melatonin has the ability to restore tissue components closer to the normal state. This result corresponds to Olukole et al. (2018).

Conclusion

The study concluded that melatonin had an encouraging effect on the prostate via the stabilization of sex hormones and the reconfiguration of histostructures.

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