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Effect of melatonin on oxidant and antioxidant status of camel cumulus-oocyte complexes and sperm

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ABSTRACT: Background/aim: Melatonin as an antioxidant had an important role in the improvement of the reproductive efficiency of animals. This study aimed to evaluate the effect of melatonin on oxidant and antioxidant parameters in camel cumulus-oocyte complexes (COCs) and sperms. Material and methods: Camel COCs and sperms were collected from ovaries and testes from the abattoir then divided into the following 4 groups: control non-treated (G1), and 3 groups treated with melatonin at low (250 µM, G2), moderate (500 µM, G3), and high (1000 µM, G4) concentration. The levels of lipid peroxidation marker malondialdehyde (MDA) and the activities of the antioxidant enzymes [catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx)] were measured in COCs and sperms of all groups.

Results: COCs and sperms treated with melatonin (G2-G4) showed significantly lower MDA levels and significantly higher activities of CAT, SOD, GPx than the untreated control samples. Among the 3 treated groups, COCs and sperms treated with high melatonin concentration exhibited lowest MD levels and highest CAT, SOD, GPx activities.

Conclusion: Melatonin could improve the reproductive efficiency of male and female camels through inhibition of reactive oxygen spices and induction of antioxidant enzymes in COCs and sperms.

Keywords: Camel, Melatonin, ROS, Antioxidant

1.Introduction

Dromedary camel is adapted to live in hot and dry climate conditions **(1)**. It supports people who live under drastic conditions by transportation and sports **(2)**. Seasonality, starvation, and bad management are all variables that limit birth rate from a single bull **(3)**. Camels that conceive in the middle or later phases of the breeding season have a minimal likelihood of conceiving in the following breeding season due to the extended gestation duration and lactation-related anestrus **(4)**. The time between calving is unusually long (about two years). In addition to the capacity to collect and process male semen and develop embryos from females, prolonged the breeding season of camel-bulls would be highly desirable, as breading late in the breeding season might reduce the intercalving period **(5)**. Compared with other domestic species, the reproductive efficiency in camelids is low. In regions with moderate climates, day-length affects seasonal breeding in mammals. Suprachiasmatic nuclei in animals keep track of the length of the day (photoperiod) and send data to the pineal gland via a multi-synaptic channel **(6;7)**. Photoperiod modulation particularly for melatonin can be utilized to promote reproductive activity during the non-breeding season by altering the pineal-hypothalamic-pituitary-gonadal endocrine axis **(8)**. Melatonin is a multifunctional chemical that plays a role in the control of many other hormones as well as a variety of physiological functions such as sleep, immunity, apoptosis, circadian rhythm coordination, blood pressure management, and antioxidant protection through its free-radical scavenging potential **(9)**

declined following a particularly bright pulse in the middle of the night, suggesting that light regulates melatonin synthesis and that camels use melatonin fluctuations to detect and integrate photoperiod variations **(10; 11)**. Fertility deteriorated during aging process due to high oxidative damage and lower endogenous antioxidant enzymes resulting in the decreased quality and number of oocytes and sperms. This study aimed to assess the impact of melatonin on oxidant and antioxidant parameters in camel cumulus-oocyte complexes (COCs) and sperms.

2.Material and methods

Collection of cumulus oocytes–complexes and melatonin-treatment

A total of 200 oocytes were aspirated from 32 ovarian specimens taking in consideration their compaction, organized cumulus cell layers and homogeneity of ooplasm according toAbdoon et al **(12)**. Grade A, cumulus oocytes–complexes (COCs) were selected from the aspirated oocytes and divided into control non-treated (G1), and 3 groups treated with melatonin at low (250 µM, G2), moderate (500 µM, G3), and high (1000 µM, G4) concentration for 24 h. Melatonin (purity 99%) was purchased from Sigma Aldrich and was dissolved in phosphate buffer saline (PBS). The G1 was treated with the vehicle (PBS).

Collection of sperms and melatonin-treatment

Sperms were collected from camel testes and epididymis which collected from a local abattoir. Sperm samples were divided into control non-treated (G1), and 3 groups treated with melatonin at low (250 µM, G2), moderate (500 µM, G3), and high (1000 µM, G4) concentration for 24 hrs.

Determination of oxidative /antioxidative markers

The levels of lipid peroxide marker malondi-aldehyde (MDA), catalase (CAT), superoxide dismutase (SOD), and glutathione peroxidase (GPx) were measured calorimetrically in COCS and perms using commercially available kits (Bio-diagnostic, Egypt).

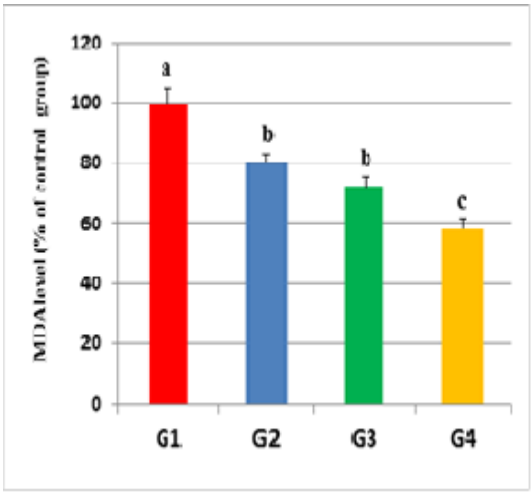
Statistical analysis

All data were expressed as means ±SEM. The statistical significance was evaluated by either one-way analysis of variance (ANOVA) using SPSS, 18.0 software, 2011 and the individual comparisons were obtained by Duncan's multiple range test (DMRT). Values were considered statistically significant when $p<0.05$.

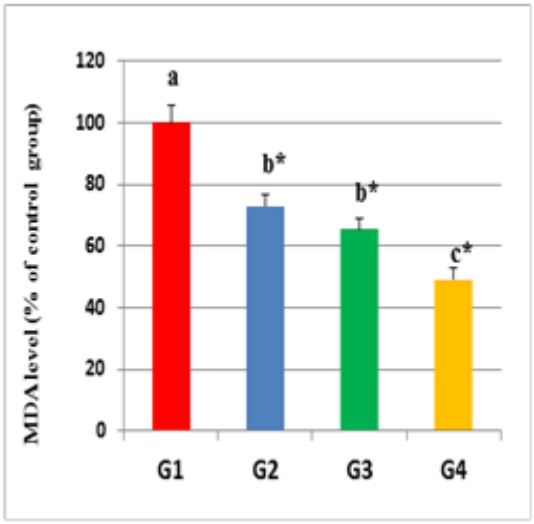
3. Results

Effect of melatonin on MDA levels in COCs and sperms

The obtained results showed significantly ($P<0.05$) lower MDA levels in COCs and sperms following treatment with melatonin at gradient concentrations of 250 µM (G2), 500 µM (G3), and 1000 µM (G4), with lowest levels in G4, as compared to the control (untreated) group (G1) (Fig. 1). No significant difference in MDA levels was noticed between G2 and G3.



COCs

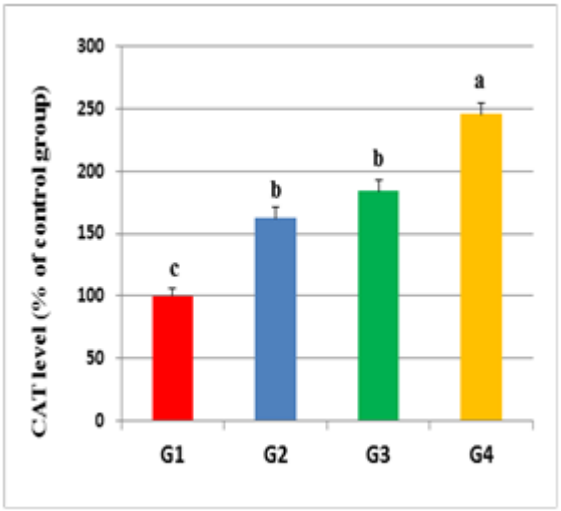


Sperms

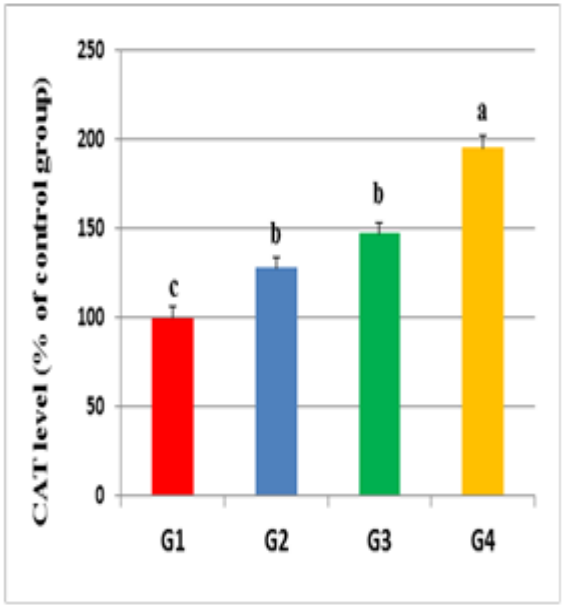
Fig. (1): Effect of melatonin treatment on MDA levels in COCs and sperms. Data were presented as mean ±SEM of three replicates

(n=3). Columns with different letters show significant differences at P<0.05.**Effect of melatonin on CAT, SOD, GPx levels in COCs and sperms**

There was significantly (P<0.05) dose dependent higher CAT, SOD, GPx levels in COCs and sperms following treatment with melatonin, with highest levels in G4, as compared to the control group (G1) (Figs. 2-4). However, no statistical difference in CAT, SOD, GPx levels was observed between G2 and G3 except for SOD in sperms which showed significant higher levels in G3.

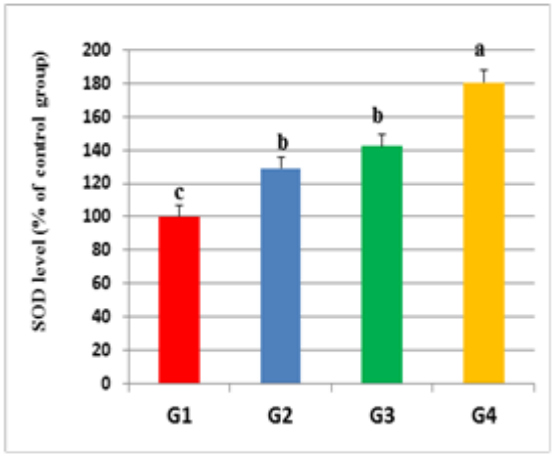


COCs

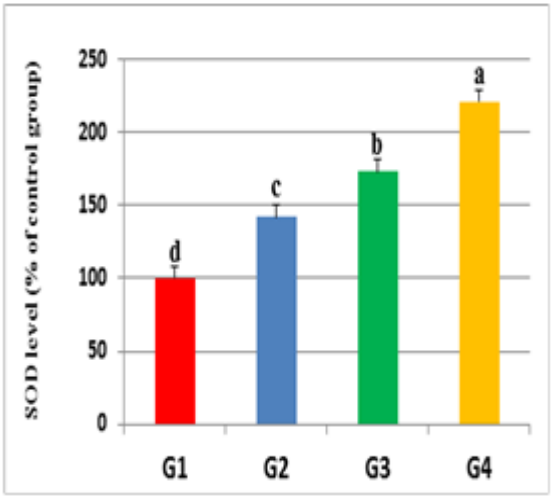


Sperms

Fig. (2): Effect of melatonin treatment on CAT levels in COCs and sperms. Data were presented as mean ±SEM of three replicates (n=3). Columns with different letters show significant differences at P<0.05.

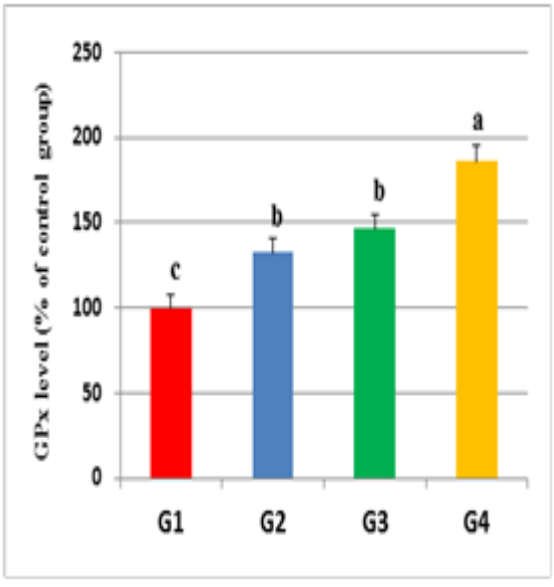


COCs

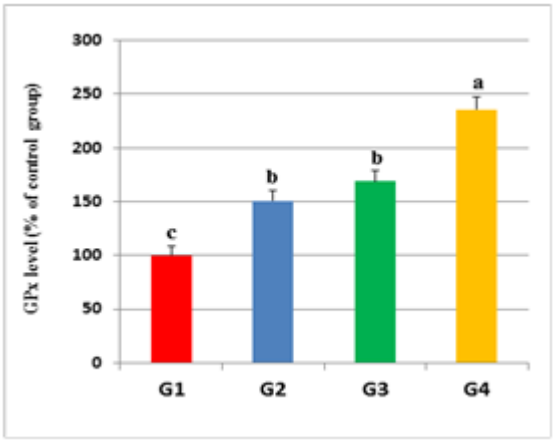


Sperms

Fig. (3): Effect of melatonin treatment on SOD levels in COCs and sperms. Data were presented as mean ±SEM of three replicates (n=3). Columns with different letters show significant differences at P<0.05.



COCs



Sperms

Fig. (4): Effect of melatonin treatment on GPx levels in COCs and sperms. Data were presented as mean ±SEM of three replicates (n=3). Columns with different letters show significant differences at P<0.05

Discussion

In the present study we evaluated the effect of melatonin on oxidant and antioxidant parameters in camel cumulus-oocyte complexes (COCs) and sperms. Our results revealed that melatonin treatment reduced level of MDA and increased level of CAT, SOD, and GPx in both the COCs and sperms. Similarly, it has been reported that melatonin enhanced the quality and development of swine oocytes in vitro, possibly through its potent antioxidant properities **(13)**. Melatonin has the ability to promote reproductive activity during the non-breeding season by altering the pineal-hypothalamic-pituitary-gonadal endocrine axis **(8)**.

Melatonin is produced by the pineal gland during the night under normal light/dark circumstances **(14)**. Its synthesis is regulated by light and camels use melatonin fluctuations to detect and integrate photoperiod changes **(10; 11)**. A number of sex hormones secreted by the body's circadian clock managing human reproductive function. A genetic signature is produced by sleep pattern that influence the synthesis, release, and metabolism of reproductive hormones produce genetic signature **(15)**. Melatonin is lipophilic, meaning it may quickly access intracellular structures and has antioxidant properties, when given at the start of reperfusion, melatonin has been proven to be more effective in preventing I/R damage **(16; 17)**, and consequently considered of antioxidant activity **(17)**.

Melatonin is an excellent antioxidant. It has a significance ability for scavenging of reactive oxygen and nitrogen species, as well as indirect detoxification through the protection of antioxidant enzymes. Melatonin also chelates transition metals implicated in the Fenton/Haber-Weiss reactions, impairing the generation of the toxic hydroxyl radical and therefore reducing oxidative stress **(17; 18)**. In vitro studies of the influence of melatonin (0.01 nM) on cumulus-oocyte and embryo development in prepubertal and adult dairy calves revealed a large percentage of blastocysts as well as a higher number of inner cell mass ICM, total cells, and trophectoderm **(19)**.

Germ cells are particularly susceptible to oxidative stress and their antioxidant capacity is lost during spermatogenesis, which is crucial for reproductive success. Melatonin as an antioxidant was found to improve gamete quality and to have potential scavenger action to prevent and reverse oxidative stress damage on fish spermatozoa **(20)**.

Conclusion

Melatonin as an antioxidant scavenger improved the antioxidant status of camel COCs and sperms as revealed by increased levels of CAT, SOD, and GPX and decreased levels of MDA. This antioxidant effect is dose dependant. Melatonin could enhance fertility through inhibition of oxidative markers and induction of antioxidant enzymes in COCs and sperms.

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