Effect of melatonin in adult quail males exposed to oxidative stress induced by H$_2$O$_2$

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Abstract

In the dark, the pineal gland secreted melatonin hormone after produced from tryptophan that is responsible for organizing many vital functions such as Wake up and sleep, circadian rhythm, Immune response and reproduction. As well as that Melatonin act as antioxidant and inflammatory.

Thirty-six of 45 days-old Japanese quail male randomly divided to four treatments nine replicates per each. The control treatment consisted of the basal diet and normal water, 2$^{nd}$ treatment of (1%) hydrogen peroxide in drinking water. 3$^{rd}$ and 4$^{th}$ treatment the ratio supplemented with (20 mg melatonin / kg diet) with or without (1%) hydrogen peroxide in drinking water. At 28 days, hematological parameters, antioxidant measurement determined and testes histological assessment. From the results noted a significant reduction in the total counting of red blood cells (RBC) and the relative weight of the testicles with a deterioration in antioxidants status, represented by a significant increase in MDA level and a significant decrease in GSH level with H$_2$O$_2$ treatment. The supplemented of melatonin led to a decrease in the total number of WBC, PCV, and the concentration of Hb. Moreover, the treatment with the use of melatonin led to an improvement in antioxidants indicators represented by an increase in the level of GSH and a decrease MDA level. As the fourth treatment did not record significant differences compared to the first treatment. Adding melatonin at 20 mg melatonin / kg diet lead to significant improvement in area of germinal layer and its thickness.

Keywords: melatonin, quail males, oxidative stress

1. Introduction

Oxygen is an element essential for life. When cells use it to production energy, free radicals are created as a consequence of (adenosine triphosphate) production from the mitochondria. Generally as a result from the cellular redox process reactive oxygen species and reactive nitrogen species products. These species play a multi role as both toxic and beneficial compounds. The balance between their two reverse effects is clearly an important aspect of life. At low or moderate levels, reactive oxygen species as and reactive nitrogen species exert beneficial effects on cellular responses and immune function. At high concentrations, they formation oxidative stress, a deleterious process that can damage cell and its organelles [1]. The body has several mechanisms to confront oxidative stress by producing antioxidants, either naturally generated in situ, or externally supplied through. The roles of antioxidants are to equation the surplus of free radicals, to protect the cells against their toxic effects and to contribute to disease prevention [2,3].

Melatonin, N-acetyl-5-methoxytryptamine, which was secreted primarily by the pineal gland in response to darkness [4]. Melatonin is a neurohormone derived from essential amino acid tryptophan [5]. Melatonin controls various physiologic processes, including circadian rhythms, mood regulation, anxiety, sleep, appetite, immune responses, cardiac functions and free radical scavenging [6]. The synthesis of melatonin was low levels during the daytime and its secretory peak at night [7]. Melatonin is act direct scavenger of free radicals. Unlike most of other radical scavengers, it is a multifunctional antioxidant. Melatonin can easily pass through cell membranes because of its high lipophilicity and hydrophilicity [8]. Melatonin is also widespread within. Unlike most small molecule biological antioxidants such as vitamin C, vitamin E, lipoic acid, etc. It was shown that melatonin reduced the formation of 8-hydroxy-2′-deoxyguanosine (8-OH-dG), a damaged DNA product, 60–70 times more effective than some Additionally, the relative position of melatonin and its metabolites in the antioxidant “pecking order” (electrochemical potential) may contribute greatly to its utility in biological systems [9]. Melatonin protects
lipids, proteins, and nuclear DNA from oxidative damage suggests that its intracellular distribution is wide (Anwar et al., 2015).

2. Materials and Methods

This study was designed to determine the effect of melatonin dietary supplementation 20 mg/kg in adult Japanese quail male 45 days concomitantly exposed to oxidative stress induced by hydrogen peroxide (1%) with drinking water for four weeks on some physiological, histological treaties and antioxidiant status.

36 adult Japanese quail male randomly divided into four treatments group nine birds/group with nine replicate (one bird per each) these birds kept in individual cages. The males were fed with commercial diets including 19.87 % crude protein and 2904 kcal ME / kg. The males were exposed to 16 h of light/day during the experimental period. The treatment group as flow (T1): Feed with standard diet and normal water. (T2): Treatment of (1%) hydrogen peroxide in drinking water. (T3): a standard diet + (20 mg melatonin / kg diet). (T4): a standard diet (20 mg melatonin / kg diet) + (1%) H2O2 in drinking water.

After 28 day, blood was collected in EDTA tubes from the jugular vein of three birds from each treatment, and used fresh blood for determination of hematological parameters. The number of total RBC (10⁶/mm³) and total WBC (10³/mm³) were determined using Natt-Herrick staining solution in a Haemocytomater chamber. Hemoglobin level (g/100 mL) was measured by the cyanmethemoglobin method and hematocrit (PCV) (%) was determined using a micro-hematocrit capillary, blood centrifuged for Glutathione (GSH) was measuring by method of [11], malondialdehyde (MDA) was estimated as method of [12]. Testes, liver and heart relatively weight determined by killed three birds by cervical dislocation.

2.1. Histology of the Testes. At the end of the study (28 days), three birds from each treatment were killed and testes were excised for histological assessment. The left testis of each male cut into serial cross-sections 5-7 mm in thickness, fixed in 10% neutral buffered formalin, embedded in paraffin, sectioned, and stained with hematoxylin and eosin for routine histological examination. Histological examination of 2 preparations of the left testis of each bird was conducted blindly under light microscope. With digital AMSCOP. Camera [13].

2.2. Statistical Analysis. All data analyzed for normal distribution using the normal option procedure of SAS software (SAS 2010). Data analyzed as a completely randomized design by the GLM procedure of SAS software. Statistical differences were established using a Duncan’s Multiple Range Test at the level of (P≤ 0.05).

3. Results and discussion

From the result of table 2, we noted significantly (p≤ 0.05) decreasing in total count of red blood cells (RBC) in quail bird treated with hydrogen peroxide (T2), as compared with control group (T1), whereas no significant differences between T1 and other treatments, in spite of rising RBC in T2 compared with T3. Treated quail birds with 20mg/kg ratio (T3) lead to significant reduction in hemoglobin concentration (Hb) and packed cells volume (PCV %) as compared with control group.

| Table 2. Effect adding melatonin in some haematological indicators for quail males exposed to oxidative stress. |
|---------------------------------|-------|-------|-------|-------|
| Treatment | T1 | T2 | T3 | T4 |
| RBC (x*10⁶)/mm³ | 4.24 ± 0.66 A | 3.21 ± 0.70 B | 4.35 ± 0.98 A | 4.76 ± 0.92 A |
| WBC (x*10³)/mm³ | 20.0 ± 2.64 AB | 23.3 ± 5.13 A | 14.3 ± 2.51B | 18.6 ± 1.15 AB |
| PCV% | 51.0 ± 2.64 A | 74.0 ± 3.60 AB | 45.3 ± 2.51 B | 50.0 ± 1.73 AB |
Table 3. Effect adding melatonin in some biological organs relative weight for quail males exposed to oxidative stress.

| Treatment | T1            | T2            | T3            | T4            |
|-----------|---------------|---------------|---------------|---------------|
| Heart %   | 0.86 ± 0.11 A | 0.86 ± 0.05 A | 1.03 ± 0.06 A | 1.08 ± 0.18 A |
| Liver %   | 1.50 ± 0.19 A | 1.79 ± 0.53 A | 1.91 ± 0.47 A | 2.14 ± 0.32 A |
| Intestine | 5.71 ± 0.55 A | 5.53 ± 1.84 A | 4.22 ± 0.32 A | 5.68 ± 1.43 A |
| Testis %  | 3.29 ± 0.31 A | 2.63 ± 0.59 B | 3.21 ± 0.41 A | 3.22 ± 0.50 A |

The same superscripts within rows mean non-significant.

a-c: Means within column with different superscripts differ significantly at (P≤ 0.05).

(T1): Feed with standard diet and normal water. (T2): Treatment of (1%) hydrogen peroxide in drinking water. (T3): a standard diet + (20 mg melatonin / kg diet). (T4): a standard diet (20 mg melatonin / kg diet) + (1%) H₂O₂ in drinking water.

The treatment with H₂O₂ created significant raising in malondialdehyde (MDA) level as compared with control group, although no differences observed between control group and others treatments. Adding 20mg melatonin /kg diet of quail birds causes significantly increasing in glutathione (GSH) level when compare it with T2 and T1. We can noted no significant differences between T4 and other treatments.

Table 4. Effect adding melatonin in some antioxidant status for quail males exposed to oxidative stress.

| Treatment | T1            | T2            | T3            | T4            |
|-----------|---------------|---------------|---------------|---------------|
| GSH m/mol | 1.64 ± 0.18 A | 1.00 ± 0.15 B | 1.47 ± 0.22 AB| 1.49 ± 0.50 AB|
| MDA       | 1.89 ± 0.15 A | 1.79 ± 0.28 A | 1.10 ± 0.06 B | 1.51 ± 0.41 AB|

The same superscripts within rows mean non-significant.

a-c: Means within column with different superscripts differ significantly at (P≤ 0.05).

(T1): Feed with standard diet and normal water. (T2): Treatment of (1%) hydrogen peroxide in drinking water. (T3): a standard diet + (20 mg melatonin / kg diet). (T4): a standard diet (20 mg melatonin / kg diet) + (1%) H₂O₂ in drinking water.

From the results of table 5, noted significant increasing in L.D, G.A and L.A. for birds treated with melatonin, even so no significant differences found in S.D and G.L.T among all treatments.

Table 5. Effect adding melatonin in testes histology for quail males exposed to oxidative stress.

| Treatment | T1            | T2            | T3            | T4            |
|-----------|---------------|---------------|---------------|---------------|
| Treats    | T1            | T2            | T3            | T4            |

The same superscripts within rows mean non-significant.

a-c: Means within column with different superscripts differ significantly at (P≤ 0.05).

(T1): Feed with standard diet and normal water. (T2): Treatment of (1%) hydrogen peroxide in drinking water. (T3): a standard diet + (20 mg melatonin / kg diet). (T4): a standard diet (20 mg melatonin / kg diet) + (1%) H₂O₂ in drinking water.
4. Discussion

The significant decrease in RBC may be due to the effect of H2O2 to cause a state of oxidative stress, by production free radicals in vital organs [13]. Including the kidney, which considered the primary site to production erythropoiesis [14]. Alternatively; it may have affected the bone marrow in the red blood cell tissue cells and thus may have reduced production of RBC. The red blood cells in birds have a low life time compared to red blood cells in mammals and this is due to high activity and high rate of operations Metabolism in birds [11].

The significant decrease in HB and PCV, may be due to their close association with the total number RBC, as [13] observed, a close relationship between the total number of red blood cells and the values of both hematopoiesis and the concentration of hemoglobin in it. The improvement in the total number of RBC may be due to the role of melatonin, which is usually associated with reducing oxidative damage [15]. One of the most important properties of melatonin that distinguishes it from other antioxidants is that its metabolites have the ability to remove reactive oxygen species (ROS) and active nitrogen types respectively. The continuous protection practiced by melatonin and its metabolites is referred to as free radical [16], which makes melatonin highly effective in protecting cells from oxidative damage even at low concentrations of melatonin [17]. The significant decline in WBC may be an indication of an improvement bird's immunity due to melatonin, which may work to improve the antioxidants status, which led to an improvement in bird's immunity due to the presence of a correlation factor between them .Perhaps the reason of low relatively weight of the testicles in birds treated with H2O2e is due to the latter's role in causing oxidative damage to the testicular tissue. Sperm production and its content of sperm [17].

The significant increase in the MDA level and significant decrease in GSH level when treated with hydrogen peroxide is consistent with [13]. To explanation GSH concentration decrease may be related to increased oxidation of GSH to the oxidized form of sulfur (GSSG), a process that catalyzes the GSH-PX enzyme [4] and that the protection afforded by GSH – pesticide to cells and tissues against lipid peroxidase depends on Re-convert the GSSG oxidized glutathione to the reduced GSH. This process relies on another enzyme (GSH-RD) that needs the Pentose shunt activity that is provided by the enzyme NADPH that works with the GSH-RD in order to re-reduce the GSSG oxidizer to the effective reduced form GSH. The low concentration of GSSG in cases of oxidative stress occurs due to a decrease in the effectiveness of Pentose shunt in these cases [18], as the G-6-PDH enzyme activity necessary for pentose phosphate shunt activity decreases reduced GSH concentration. Thus, formation NADPH decreases.

One such of melatonin activity is antioxidant capacity Attributed to two side chains, a 5-methoxy group and 3-amide group and an indoleamine. [19]. Melatonin and its metabolites can easily pass through cell membranes, because of its high lipophilicity and hydrophobicity; this stands out it's important in direct and indirect antioxidant actions [20]. By a direct scavenging to detoxify reactive oxygen species (ROS), nitrogen species (NS) and free radical, melatonin protects many biomolecules against damage caused by these oxidizes. Under oxidative stress conditions, glutathione concentration can be reduced in many cells [21]. Melatonin preserve the activities of enzymes that boost intracellular levels of reduced GSH. A main effect of melatonin in reducing oxidative stress done through recycling of glutathione (GSH). Glutathione is oxidized to its disulphide glutathione (GSSG), which is then quickly reduced back to GSH by Glutathione reductase (GR). Melatonin stimulate these enzyme one of the antioxidant activities. The regulation of GSH/GSSG balance attributed to ability of melatonin to modulating enzyme activities seems to involve an action of melatonin at a nuclear binding site [22], as a response to melatonin increases many GSH-metabolizing enzyme, i.e., CAT [18]. Moreover, melatonin regulate glutathione production via stimulate gamma-glutamylcysteine synthetises, thus glutathione levels height significantly [23], so that one melatonin molecule has the capacity to scavenge up to 10 ROS versus the classic antioxidants(vitamin C, vitamin E ...) that

| S.D  | 252.6±1.29 A | 258.0±1.64 A | 263.0±4.4 A | 252.8 ±4.1A |
|------|--------------|--------------|-------------|-------------|
| L.D  | 139.3±5.7 B  | 152.9±2.8 A  | 124.3±2.7 C | 137.3±4.0 BC|
| G.A  | 34830.6±736.4 B | 33914.5±1470.8 B | 42402.9±2358.6 A | 35383.9±1935 B |
| L.A  | 15293.3±1254.8 B | 18365.3±686.3 A | 12145.5±528.8 C | 14834.3±893.2 BC |
| G.L.T| 113.3±4.4 B  | 105.1±4.8 B  | 139.2±7.1 A | 115.5±6.0B |

- The same superscripts within rows means non-significant.
- Means within column with different superscripts differ significantly at (P≤ 0.05).
- (T1): Feed with standard diet and normal water. (T2): Treatment of (1%) hydrogen peroxide in drinking water. (T3): a standard diet + (20 mg melatonin / kg diet). (T4): a standard diet (20 mg melatonin / kg diet) + (1%) H2O2 in drinking water.
- S.D: seminiferous tubules diameter, L.D: seminiferous tubules lumen diameter, G.A area of seminiferous tubules lumen, GL.T: germinal layer thickness.
scavenge one or less ROS [13]. Additionally, the relative position of melatonin and its metabolites in the antioxidant “pecking order” (electrochemical potential) may contribute greatly to its utility in biological systems [9]. Melatonin protects lipids, proteins, and nuclear DNA from oxidative damage suggests that its intracellular distribution is wide [10]. Melatonin turned out to be considerably more efficient than the majority of its naturally occurring structural analogs, indicating that the substituents of the indole moiety strongly influenced reactivity and selectivity [16]. This positive effect in antioxidant status may be related to improve the testis histological indicators this agreement with [13,24], when founding positive correlation between antioxidant statues and histological structure of testes.

Conclusion

These results lead us to suggest, that the addition of melatonin to the diets of quail exposed to oxidative stress. Improvement most blood characteristics and antioxidants parameters accompanied by an improvement in the testicle histological structure.

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