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Ameliorative Effects of Melatonin on Exercise-induced Oxidative Stress and Haematological Response of Untrained Arabian Stallions Following a Race Of 2000 m

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ABSTRACT

We performed this study to evaluate the effects of melatonin on oxidative stress and haematological responses following an exercise of 2000 m at maximum speed. Eighteen untrained, clinically healthy stallions of the Arabian breed with a mean body weight of 401 ± 32.11 kg (395–404 kg) and an age range of 5.7–0.54 years (5–6 years) were used in this research. The subjects were divided into three groups of six stallions each. Group I (MTEX) was treated with melatonin at a dose rate of 0.03 mg/kg orally once daily for one month. Group II (NMTX) was not administered melatonin but exercised, while the last group (OMTX) was neither administered melatonin nor exercised. The results obtained show that post-exercise, the biomarkers of oxidative stress evaluated were significantly lower ($p < 0.05$) in the MTEX group than in the NMTX group. The leucocyte count, neutrophil counts, and the ratio of neutrophil to lymphocyte were higher ($p < 0.05$) in the NMTX group than in the MTEX group. Furthermore, it was recorded that packed cell volume and the total erythrocyte count were statistically higher ($p < 0.05$) in the MTEX group than in the NMTX group. Therefore, we concluded that melatonin ameliorated oxidative stress and some haematological parameters will be beneficial to horses subjected to the stress of exercise.

Keywords

Arabian stallion, haematological parameters, melatonin, oxidative stress

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Abbreviations

MTEX: Group treated with melatonin before exercise
OMTX: Not treated with melatonin but exercised
NMTX: Not treated with melatonin, not exercised
PUFA: Polyunsaturated fatty acid

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Introduction

The horses are used for a variety of activities, but they need to be extensively trained and exercised for easy restoration and their general well-being [1,2]. Exercise increases the body's need for oxygen, alters metabolic load, and increases the heart rate alongside other physiological responses to withstand the demand for exercise [3,4]. Exercise Physiology of Horses has been widely studied, and research has demonstrated that exercise improves cognitive abilities and health status [5.6]. However, exercise can be a source of stress, and stress is a term used to describe a situation, circumstance, or stimulus that can endanger or affect an organism's homeostasis [7, 8, 9]. When stress is not managed or prevented, it can become maladaptive, impairing the physiological process of the body [10].

Two interconnected branches of the autonomic nervous system that make up the physiological stress response are the sympathetic adrenal medullary network, which reacts quickly to stress and facilitates the release of adrenaline into the bloodstream and ultimately into brain regions, and the slower hypothalamic pituitary adrenal network, which facilitates the release of glucocorticoids [11,12, 13]

Increase in oxidative stress has been defined as an imbalance in the body's oxidant-to-antioxidant ratio. It has been showen that in horses, oxidative stress levels rise, and their antioxidant status changes during endurance and high-intensity competition [7, 14, 15]. All antioxidant substances—vitamins, minerals, enzymes, and proteins—must either be synthesized by the body or obtained through diet [16]. As a result, the oxidative stress and antioxidant state of the horse athlete are both influenced by nutrition and exercise intensity [17, 18, 19]. During endurance and competitive exercise events, horses have demonstrated increased oxidative stress and changes in their antioxidant status [7, 20]. Melatonin and its metabolites, which are potent anti-inflammatory agents and antioxidants, protect mitochondrial integrity by scavenging reactive oxygen species and reactive nitrogen species [21,22]. Melatonin and its derivatives induce several antioxidant enzyme activities. Thus, melatonin is necessary to slow down the process of aging and maintain the cellular redox equilibrium [23].

The objective of this study is to determine the effects of melatonin on biomarkers of oxidative and haematological parameters of stallions of the Arabian breed subjected to an exercise of 2000 m.

Results

The biomarkers of oxidative stress are shown in Table 1. The activity of superoxide dismutase obtained MTEX group (2.49 ± 0.14 u/ml) post-exercise was statistically lower ($p < 0.05$). The activity of the enzyme catalase in the NMTX group (10.05 ± 5.43 u/ml) post-exercise was statistically higher ($p < 0.05$) than the value obtained in the MTEX group (6.04 ± 0.43 u/ml) after exercise. The activity of the enzyme glutathione peroxidase was lower ($p < 0.05$) in the MTEX group than in the NMTX group after exercise (278.13

Table 1.
Biomarkers of Oxidative Stress

Parameters	Time	OMTX	MTEX	NMTX
Superoxide dismutase (u/ml)	Pre-exercise	1.77 ± 0.33	1.62 ± 0.21	2.57 ± 0.37
	Post-exercise	2.41 ± 0.54	2.49 ± 0.14a	8.57 ± 4.78b
Catalase (u/ml)	Pre-exercise	4.57 ± 0.41	4.97 ± 0.23	4.74 ± 1.531
	Post-exercise	4.38 ±0.37	6.04 ± 0.43a	10.05 ± 5.43b
Glutathione peroxidase (U/L)	Pre-exercise	267.13 ± 5.66	259.90 ± 5.32	267.82 ± 7.73
	Post-exercise	264.37 ± 6.31	278.13 ± 8.43a	324.05 ± 19.07b2
Glutathione reductase (U/L)	Pre-exercise	0.38 ± 0.02	0.44 ± 0.16	0.45 ± 0.03
	Post-exercise	0.41 ± 0.03	0.87 ± 0.34	1.58 ± 1.33
Malondialdehyde (µmol/L)	Pre-exercise	5.58 ± 0.27	5.01 ± 0.34	5.72 ± 1.061
	Post-exercise	5.42 ± 0.49	7.26 ± 0.56a	10.24 ± 5.77b

a,b Means for the same row having different superscript letters are significantly ($P < 0.05$) different
Key: OMTX: Not administered with melatonin, not exercised
MTEX: Administered with melatonin, then exercised
NMTX: Not administered with melatonin but exercised

± 8.43 U/L and 324.05 ± 19.07 U/L, respectively). The concentration of malondialdehyde after exercise in the MTEX group (7.26 ± 0.56 µmol/L was lower ($p < 0.05$) than the value obtained in the NMTX group (10.24 ± 5.77 µmol/L).

Table 2 shows that the total count of leucocytes obtained in the MTEX group (4.83 ± 0.14 $\times 10^9$/L) was significantly lower ($p < 0.05$) than the value obtained in the NMTX group (7.31 ± 0.86 $\times 10^9$/L). The neutrophil count recorded in the NMTX group after exercise (5.34 ± 0.85 $\times 10^9$/L) was significantly higher ($p < 0.05$) than the count recorded in the MTEX group after exercise (3.34 ± 0.11 $\times 10^9$/L).

The ratio of neutrophil to lymphocyte calculated in the NMTX group after exercise (3.97 ± 1.64) was statistically higher ($p < 0.05$) than the value calculated in the MTEX group (2.08 ± 0.35) post-exercise.

Table 3 shows the erythrocyte parameters. The erythrocyte count obtained in the MTEX group (12.22 ± 6.74 $\times 10^{12}$/L) post-exercise was statistically higher ($p < 0.05$) than the value recorded in the NMTX group (8.72 ± 0.44 $\times 10^{12}$/L) after exercise. The PCV obtained in the NMTX group was statistically lower ($p < 0.05$) than the value recorded in the MTEX group (26.87 ± 0.76 % and 32.13 ± 6.25 %, respectively).

Table 2.
Biomarkers of Oxidative Stress

Parameters	Time	OMTX	MTEX	NMTX
Leucocytes ($\times 10^9$/L)	Pre- exercise	5.13 ± 0.32	4.21 ± 0.23	5.41 ± 0.37
	Post-exercise	5.07 ± 0.28	4.83 ± 0.14a	7.31 ± 0.86b
Neutrophils ($\times 10^9$/L)	Pre-exercise	2.77 ± 0.08	2.53 ± 0.34	2.83 ± 0.46
	Post-exercise	2.83 ± 0.17a	3.34 ± 0.11a	5.34 ± 0.85b
Lymphocytes ($\times 10^9$/L)	Pre-exercise	1.68 ± 0.23	1.82 ± 0.25	1.72 ± 0.21
	Post-exercise	1.69 ± 0.33	2.68 ± 0.34	1.88 ± 0.42
Monocytes ($\times 10^9$/L)	Pre-exercise	0.13 ± 0.03	0.13 ± 0.05	0.17 ± 0.02
	Post-exercise	0.11 ± 0.02	1.07 ± 0.16	0.18 ± 0.04
Neutrophil/Lymphocyte Ratio	Pre-exercise	1.78 ± 0.14	1.21 ± 0.32	1.86 ± 0.78
	Post-exercise	1.81 ± 0.16	2.08 ± 0.35a	3.97 ± 1.64b.

a,b Means for the same row having different superscript letters are significantly ($P < 0.05$) different
Key: OMTX: Not administered with melatonin, not exercised
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Table 3.
Erythrocyte indices of stallion

Parameters	Time	OMTX	MTEX	NMTX
Erythrocytes ($\times 10^{12}$/L)	Pre-exercise	5.56 ± 0.62	7.12 ± 0.73	5.98 ± 0.23
	Post-exercise	5.78 ± 0.53	12.22 ± 6.74a	8.72 ± 0.44b
Packed Cell Volume (%)	Pre-exercise	26.11 ± 0.93	32.13 ± 6.25a	26.87 ± 0.76b
	Post-exercise	29.65 ±1.02	63.42± 13.67a	55.42± 4.74b
Haemoglobin (g/dl)	Pre-exercise	8.55 ±1.21	8.71 ± 2.41	7.71 ± 0.36
	Post-exercise	8.62 ± 1.09	11.53 ± 0.74	10.83 ± 0.68

a,b Means for the same row having different superscript letters are significantly ($P < 0.05$) different
Key: OMTX: Not administered with melatonin, not exercised
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Discussion

Exercise has been reported to be beneficial to horses [24] but extreme exercise without requisite dietary antioxidant supplementation may be deleterious. This is because, during intense exercise, it has been demonstrated that there are increases in oxidative stress response and altered antioxidant status in horses [17]. In this study, we observed that the biomarkers of oxidative stress were higher in the NMTX group than in the MTEX group, indicating a higher level of oxidative stress process in the NMTX group.

Melatonin use has been demonstrated to considerably ameliorate oxidative stress indicators [25]. However, the effect of melatonin on certain oxidative stress markers, such as during exercise in horses, has not been well reported in the literature. In this study, we observed that most of the biomarkers of oxidative stress studied were significantly lower in the MTEX group post-exercise. Melatonin improves the antioxidative potential of the cell by enhancing the synthesis of enzyme-based antioxidants like superoxide dismutase, glutathione peroxidase, and also the enzymes that are precursors in the synthesis of glutathione [26], thereby quenching oxygen free radicals such as superoxide radical, hydroxyl radical, peroxyl radical, and peroxynitrite anion. Melatonin often improves the synthesis of the antioxidative enzymes' messenger RNAs (27) and also chelates transition metal ions and shields cellular membranes from damage. This chain of events helps to explain how melatonin lowered oxidative stress in the MTEX group (28). This is also similar to the findings of Kruk et al. (27).

Due to its interaction with lipids, melatonin can stabilize the erythrocyte membrane, thereby prolonging the life of cells (29). This could explain the higher erythrocyte count recorded in the MTEX group. Intense exercise is known to increase reactive oxygen species, which results in the lysis of the erythrocytes (30), especially in horses not supplemented with antioxidants. This phenomenon may explain the lower erythrocyte count obtained in the NMTX group post-exercise. This is similar to the finding of Krokosz et al. (29). This is because, lipid peroxidation, a phenomenon where oxidants such as free radicals destroy lipids with carbon-carbon double bonds, specifically polyunsaturated fatty acids (PUFAs) occur in the membrane of the erythrocyte making their membranes fragile.

The higher leucocyte and neutrophil count recorded in the NMTX group indicates that there was a higher inflammatory response in the group due to exercise. On the other, the lower counts in the MTEX group suggest a protective role of melatonin as melatonin has been reported to possess a potent anti-inflammatory function [31]. The ratio of neutrophil to lymphocyte (a biomarker of inflammation) was higher in the NMTX group compared to the MTEX group post-exercise, further substantiating the anti-inflammatory effects of melatonin.

Materials & Methods

Animals

For this experiment, a total of 18 clinically healthy Arabian stallions with mean body weights between 401 ± 32.11 kg (395-404 kg) and ages between 5.7 ± 0.54 years (5 - 6 years) were used. The stallions were primarily used for pleasure riding and were acquired from the Royal horse stable. They lived in a stable with concrete floors, corrugated iron roofs, wooden ceilings, and cement brick walls. Before the experiment, they had a four-week pre-conditioning period. They were given hay as their primary food source, with concentrate (groundnut bran) as a supplement, and water was available at all times.

The stallions were divided into three groups of six stallions each. Group I (MTEX) was administered with melatonin once daily for one month before exercise. Group II (NMTX) was not administered melatonin but exercised while the last group (OMTX) was neither administered melatonin nor exercised.

Experimental design

The stallions in MTEX and NMTX groups were conditioned to exercise before the experiment began by putting them through a calibrated exercise protocol every day for two weeks.

Administration of melatonin

Melatonin was purchased from Twinlab in Hauppauge, New York, USA. Melatonin was given to each stallion in the MTEX group at a dose of 0.03 mg/kg body weight orally once daily for one month. The medication was given to the stallions after being dissolved in water and aspirated using the appropriate syringe daily for a month.

Exercise protocol

Each stallion in the MTEX and NMTX group was mounted by a trained rider, with a weight of approximately 70 kg. The stallions were then subjected to a race of 2000 m on a standard race track at maximum speed.

Blood sampling

Each stallion had 10 mL of blood collected from the jugular vein before and after exercise. Exactly 5 ml of the blood samples were dispensed into sample bottles containing potassium ethylenediaminetetraacetic acid, an anticoagulant for haematological analyses, while the remaining 5 ml were collected in plain sample bottles for serum harvesting, which was used for the evaluation of biomarkers of oxidative stress. The blood samples collected were transported to the laboratory in a Coleman box containing ice and analyzed immediately.

Analyses of samples

A standard veterinary automated analyzer (KT-6610 VET, Jiangsu, China) was used to determine the total count of erythrocytes, the total count of leucocytes, haematocrtit value (packed cell volume), and hemoglobin concentration.

The concentration of malondialdehyde, superoxide dismutase, glutathione peroxides, and catalase activities was determined spectrophotometrically using their respective ELISA kits (Cayman Chemicals, Ann Arbor, Michigan, USA). The spectrophotometer manufactured by Spectronic-20, Philip Harris Limited, Shenstone, UK, was used for the analyses.

Data analyses

This study's data were analyzed using one-way analysis of variance (ANOVA), followed by Tukey's posthoc test, and expressed as Mean ± SEM. Values of $P < 0.05$ were regarded as significant. The studies were performed using the program GraphPad Prism (version 5.0).

Conclusions

According to our findings, we concluded that melatonin ameliorates biomarkers of oxidative stress and some haematological parameters following exercise in stallions. Therefore, supplying melatonin as a potent antioxidant is crucial for horses before strenuous exercise.

Authors' Contributions

ASA: Conceptualization, writing the original draft, and carrying out the experiments

DAA: Data analyses, and project administration

CON: Manuscript revision, funding, and carrying out the experiments

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Competing Interests

The authors hereby declare that there was no conflict of interest.

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