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Daily variation in antioxidant enzymes and lipid peroxidation in lungs of a tropical bird *Perdicula asiatica*: Role of melatonin and nuclear receptor RORα

R.K. Kharwar, C. Haldar *

Pineal Research Laboratory, Department of Zoology, Banaras Hindu University, Varanasi, 221 005, India

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ABSTRACT

The wild animals are exposed in nature to more oxidative stress than any laboratory animals. Studies on oxidative stress of brain, liver and kidney are quite common while very less reports are available on respiratory system when it is the most susceptible organ to various stressors. We checked the oxidative stress of lung tissue of a wild seasonally breeding bird *Perdicula asiatica* by noting down the daily variation in antioxidant enzymes (superoxide dismutase and catalase) levels, lipid peroxidation in terms of malondialdehyde level and total antioxidant status during reproductively active (RAP) and inactive phase (RIP). On the other hand melatonin has been accepted as free radical scavenger acting via receptor (nuclear receptor) or non receptor pathway. To pin point the role of melatonin in regulation of antioxidant enzymes via non receptor mediated pathway in lungs of bird, we checked variation in the nuclear melatonin receptor RORα. Antioxidant enzymes (superoxide dismutase and catalase) exhibited a marked 24 h rhythm in lungs being high during night time and coincided almost with the peak of melatonin and total antioxidant status where as malondialdehyde level and nuclear receptor RORα showed inverse relationship with all the above mentioned parameters. These findings suggest that melatonin might be acting as an antioxidant for the free radical load of lung tissue of a tropical bird *P. asiatica* and its action might be via nuclear receptor RORα.

1. Introduction

Avian species have to survive in diverse environmental conditions which keep on changing from place to place and time to time. Lung is a target organ for different kind of pathogens which may lead to several diseases in the birds. The wild birds are exposed to more oxidative stress than any laboratory avian species as their respiratory system is more frequently exposed to various pollutants. Such an oxidative load/stress on the lungs of any avian species has never been recorded.

Melatonin, an indolamine synthesized mainly in the pineal gland of vertebrates and also in retina and gastrointestinal tract is known for its immune stimulation (Maestroni, 1993; Bubenik, 2002; Singh and Haldar, 2007a,b) and also for free radical scavenging activity (Hardeland et al., 1993; Allegra et al., 2003; Sharma et al., 2008). The discovery of melatonin as a direct free radical scavenger (Tan et al., 1993) and as an indirect antioxidant via its stimulatory actions on antioxidative enzymes (Reiter et al., 2000; Rodriguez et al., 2004; Sharma et al., 2008) increased our interest to use this neurohormone for experimental studies on oxidative stress in birds. Such an activity has never been checked in any wild avian species though there are some reports in chickens (Albarran et al., 2001) and mammals (Pieri et al., 1995; Benot et al., 1998). An endogenous rhythm of melatonin, total antioxidant status and superoxide dismutase activity in several tissues of chick and their inhibition by light has already been noted (Albarran et al., 2001; Martin et al., 2003). Those changes with time in chick suggest that it might be dependent on the circadian melatonin rhythm (Albarran et al., 2001). Melatonin regulates antioxidant enzyme gene expression and activity with activation of Mel_{1a}/Mel_{1b} receptors, via G inhibitory (Gi) protein (Mayo et al., 2002; Rodriguez et al., 2004). Localization and daily variation of melatonin receptor types (Mel_{1a} and Mel_{1b}) and its correlation with lung associated immunity in *Perdicula asiatica* during two reproductive phases has already been established (Kharwar and Haldar, 2011a,b). The melatonin receptor variation and its relation with oxidative status of the lung of any avian species have not been reported. On the other hand possibility of the involvement of nuclear receptor ROR/RZR in regulation of antioxidative enzymes could not be neglected (Pablos et al., 1997). The participation of ROR/RZR in the regulation of immune processes, central nervous system differentiation, and possibly, the modulation of lipid metabolism has been studied (Smirnov, 2001). Moreover, there are only a few studies *in vivo* on the role of nuclear receptors in the realization of the multiple functions of melatonin (Missbach et al., 1996; Weisenberg et al., 1998). Perhaps this is partly due to the double nature type of ROR/RZR activity: constitutive and ligand dependent. Jetten (2009) suggested that RORα function as subcomponent of the circadian oscillator and regulates various physiological processes which includes lipid and genobiotic

* Corresponding author. Tel.: +91 542 2307149x125; fax: +91 542 2368174, 2575093.
E-mail address: chaldar2001@yahoo.com (C. Haldar).

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metabolism. RORα$_1$ has been reported to provide the protection to oxidative stress induced apoptosis in cortical neurons (Boukhtouche et al., 2006). So far, neither localization nor daily variations in level of RZR/ROR receptors were shown in lung of any avian species as reported in mouse lung (Naji et al., 2004)

We therefore, noted the: (1) daily periodicity of antioxidant enzymes (superoxide dismutase and catalase) in the lungs along with circulatory level of melatonin, (2) lipid peroxidation in term of thiobarbituric acid reactive substances (TBARS) to check the oxidative stress in lung and (3) RORα receptor expression in isolated total lung protein. To delineate the action of melatonin on oxidative stress and RORα receptor expression, melatonin injection were also given to birds at evening hours during two reproductive phases active and inactive.

2. Materials and methods

2.1. Animal model and experimental design

The experiment was conducted in two sets (set-I and set-II). For the set-I of experiment, 72 healthy male birds, *P. asiatica* were collected from local areas of Varanasi and maintained in an open air aviary during two reproductive phases. They were then randomly selected and divided into six groups of 12 animals in each. Randomly selected birds were acclimatized in laboratory condition for a fortnight before conducting the experiment and maintained in a room exposed to ambient conditions (RAP; June: Photoperiod approximately 14L: 10D; Maximum and minimum temperature 37 ± 5 °C and 26 ± 5 °C; humidity approximately 65% and RIP; January: photoperiod approximately 11L: 13D; Maximum and minimum temperature 15 ± 5 °C and 6 ± 3 °C; humidity approximately 90%) with water and food *ad libitum*. Birds were sacrificed by decapitation at every 4 h intervals over a 24 h cycle under the natural light condition. During the dark phase of 24 h cycle the birds were sacrificed under dim red light and samples were processed.

The set-II of experiment had 14 healthy male birds divided in two groups of 7 birds in each. First group was treated with normal saline and served as control (Con) while the second group was treated with melatonin (Mel) at evening h (17:00–17:30 h) at a dose of 25 μg/100 g. B.wt./day (at concentration of 25 μg/100 μL; Singh and Haldar, 2005). Treatment was carried out for 20 days during both the reproductive phases. After completion of treatment the birds were sacrificed in the evening hours for sample (lung tissue and blood) collection.

Present experiment was performed in accordance with institutional practice and within the framework of revised animals Act of 2007 of Govt. of India on animal welfare (Committee for the Purpose of Control and Supervision of Experiments on Animals; CPCSEA). All the chemicals used were purchased from Sigma–Aldrich Chemicals, St Louis, MO, USA.

2.2. Hormonal analysis

For the first set of birds blood from pectoral vein was collected at 4 h interval over a cycle of 24 h during both the reproductive phases (RIP and RAP). For second set of birds (control and treated with melatonin) blood from pectoral vein was collected in the evening hours during sacrifice (17:00–17:30 h). After centrifugation the plasma was collected and stored at −20 °C. To carry out hormonal evaluation of plasma melatonin, radioimmunoassay was performed following the modified method of Rollag and Niswender (1976). The validation of RIA was performed as described earlier (Haldar and Rai, 1997; Sudhakumari et al., 2001). The sensitivity for melatonin RIA was 18–20 pg/mL. The intra and inter assay variation for melatonin was 9 and 15% respectively and the recovery was 92%.

2.3. Lipid peroxidation (LPO) assay by thiobarbituric acid reactive substances (TBARS) level estimation

After sacrifice of birds, the lung tissue was dissected out on a sterile watch glass placed in ice box, cleaned from adherent tissues and processed immediately for estimation of lipid peroxidation. Lung tissues of set-I and set-II experimental birds were weighed and homogenized in a ten fold excess of 20 mM Tris–HCl buffer (pH 7.4) and the 10% homogenates were centrifuged for 15 min at 3000 g at 4 °C. The supernatant was subjected to thiobarbituric acid (TBA) assay by mixing with 8.1% sodium dodecyl sulfate (SDS), 20% acetic acid, 0.8% TBA and then digested it for 1 h at 95 °C (Sharma et al., 2008). The reaction mixture was immediately cooled in running water, vigorously shaken with 2.5 mL of n-butanol and pyridine reagent (15:1) and centrifuged for 10 min at 1500 g (Ohkawa et al., 1978). The absorbance of the upper phase was measured at 534 nm. Total thiobarbituric acid reactive substances (TBARS) were expressed as malondialdehyde (MDA; nmol/g tissue weight) taking 1,1,1,1-tetraethoxy propane (TEP) as standard. The standard curve was calibrated using 10 nM TEP.

2.4. Antioxidative enzyme activity

2.4.1. Superoxide dismutase activity

Superoxide dismutase (SOD; EC 1.15.1.1) activity was assayed following the method of Das et al. (2000). Just after sacrifice, 10% homogenates of all lung tissues from set-I and set-II birds were prepared in 150 mM phosphate buffered saline (PBS, pH 7.4) and centrifuged for 30 min at 12,000 g at 4 °C. The supernatant was again centrifuged for 60 min at 12,000 g at 4 °C and then processed for enzymic activity based on a modified spectrophotometric method using nitrite formation by superoxide radicals. A 0.5 mL of homogenate was added to 1.4 mL of reaction mixture comprised of 50 mM phosphate buffer (pH 7.4), 20 mM L-methionine, 1% (v/v) Triton X-100, 10 mM hydroxylamine hydrochloride, 50 mM ethylene diamine tetraacetic acid (EDTA) followed by a brief pre-incubation at 37 °C for 5 min. Next, 0.8 mL of riboflavin was added to all samples along with a control containing buffer instead of sample and then exposed to two 20 W fluorescent lamps fitted parallel to each other in an aluminium foil coated wooden box. After 10 min of exposure, 1 mL of Greiss reagent was added and absorbance of the color formed was measured at 543 nm. One unit of enzyme activity is defined as the amount of SOD inhibiting 50% of nitrite formation under assay conditions.

2.4.2. Catalase activity

Catalase (CAT; EC 1.11.1.6) activity was measured following the procedure of Sinha (1972). This method is based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of H_2O_2, with the formation of perchromic acid as an unstable intermediate. The chromic acetate thus produced is measured calorimetrically. The catalase preparation is allowed to split H_2O_2 for different periods of time. The reaction is stopped at a particular time by the addition of dichromate/acetic acid mixture and the remaining H_2O_2 is determined by measuring chromic acetate calorimetrically after heating the reaction mixture. There is production of green color at the end of the process. Immediately after sacrifice, 20% homogenate of lung tissue from set-I and set-II birds was prepared in PBS (10 mM; pH = 7.0) and then centrifuged at 12,000 g for 20 min at 4 °C. Supernatant was taken for enzyme estimation. 5 mL of PBS was added to 4 mL of H_2O_2 (200 mM) and then 1 mL of enzyme extract was added. After 1 min 1 mL of this solution was taken in a tube and 2 mL of K_2Cr_2O7 (5%) solution was added. Then it was boiled for 10 min and absorbance was measured at 570 nm. The activity of CAT was expressed as amount of H_2O_2 degraded per minute.

2.5. Total Antioxidant Status (TAS)

The free radical scavenging activity of antioxidants for 2,2′-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS) radical cations was measured according to the method of Re et al. (1999). This method measures the antioxidant activity determined by decolorization assay of the ABTS radical cation, through measuring the reduction of the radical cation as the percentage inhibition of absorbance at 734 nm. A stock solution of ABTS radical cations was prepared one day before the assay by mixing 5 mL of 7 mM ABTS with 1 mL of 14.7 mM potassium persulfate, followed by storage in the dark at room temperature. The stock solution of ABTS radical cations was diluted with water or ethanol. ABTS radical cation was generated by oxidation of ABTS with potassium persulfate. 2.95 mL of ABTS cation solution was mixed with 50 μL of 10% homogenate of fresh lung tissue from both set-I and set-II birds and the decrease in absorbance was monitored for 10 min at particular interval of time at 734 nm.

2.6. Western blot analysis

In brief, 10% homogenate of lung tissue of set-I and set-II birds was prepared in radioimmunoprecipitation assay buffer (RIPA buffer) (1% (v/v) Igel CA-630, 0.5% (w/v) sodium deoxycholate, 0.1% (w/v) sodium dodecyl sulfate (SDS) in phosphate-buffered solution (PBS) containing aprotonin and sodium orthovanadate). Tissue homogenates were centrifuged for 20 min at 16,000 g and supernatants were collected to assay protein expression. Determination of protein content in lung tissue isolate was carried out by using the method of Bradford (1976). Samples containing 120 μg of protein were resolved by 8% (w/v) SDS-PAGE along with Spectra Multicolor Broad Range Protein Marker (SM1841) to note the immunoreactive bands and were followed by electrotransfer to a nitrocellulose membrane. Immunodetection was carried out using RORα receptor antibodies (RORα, sc-6062, Santa Cruz Biotechnology, CA, USA, dilution 1:200) for 5 h at room temperature followed by horseradish peroxidase conjugated secondary antibody (anti-goat donkey IgG), which was detected using chemiluminescence (ECL) system (Amersham, Buckinghamshire, UK). Densitometric analysis of protein level was performed with the help of Scion Image analysis software (www.scioncorp.com). The ratio of density was calculated after normalization with β actin and was expressed as % control value.

2.7. Statistical analysis

Statistical analysis of the data was performed with one-way ANOVA followed by Student Newman–Keuls' Multiple Range test. Results are expressed as mean ± SEM. The differences were considered significant with $P<0.05$ and highly significant when $P<0.01$ (Bruning and Knitz, 1977). Microsoft Excel program was used for statistical calculations and data presentation.

3. Results

3.1. Daily variation

3.1.1. Melatonin level

Daily variations in plasma melatonin level during RIP and RAP were presented in Fig. 1a. Melatonin concentration was highest at 22:00 h and minimum at 06.00 h during RIP and highest at 02:00 h and minimum at 06:00 h during RAP (Fig. 1a).

3.1.2. Lipid peroxidation (LPO) assay by TBARS level estimation

Malondialdehyde level was highest at 18:00 h during both the reproductive phases while it was lowest at 2:00 h during RAP and at 22:00 h during RIP (Fig. 1b,c).

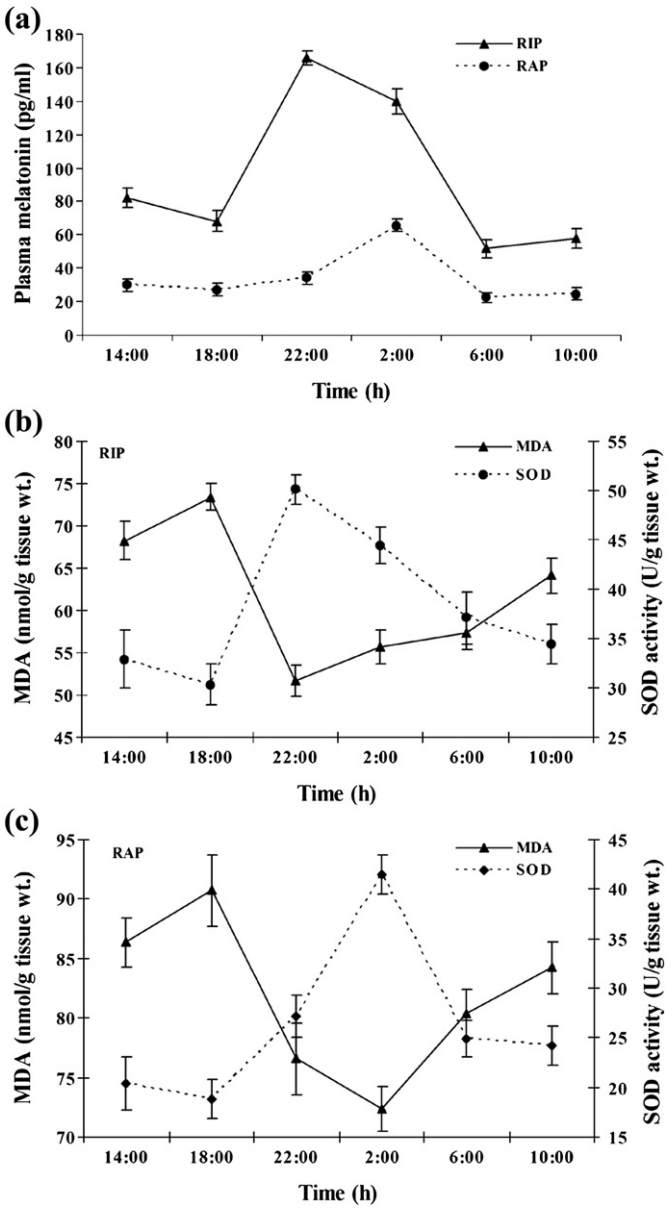

Fig. 1. (a) Radioimmunoassay showing 24 h rhythm of plasma melatonin concentrations in reproductively inactive (RIP) and active (RAP) phases of Indian Jungle bush quail, *P. asiatica*. (b) 24 h rhythm of SOD and MDA level in the lung of birds in groups at different time points (14:00, 18:00, 22:00,2:00, 6:00 and 10:00) during reproductively active phase (RAP; June) and (c) 24 h rhythm of SOD and MDA level in the lung of birds in groups at different time points (14:00, 18:00, 22:00,2:00, 6:00 and 10:00) during reproductively inactive phase (RIP; December). Data are expressed as the Mean ± SEM, $n=5$ for each group.

3.1.3. Antioxidative enzymes activity

SOD activity in lung tissues exhibited a marked 24 h rhythm. SOD level was maximum at 2:00 h in RAP and 22:00 h in RIP and minimum concentration was noted at 14:00 h (Fig. 1b,c) during both the reproductive phases.

Catalase also shows a regular rhythm over a 24 h cycle. Its maximum activity was noted at 2:00 h in RAP and 22:00 h in RIP and minimum activity was found at 14:00 h (Fig. 2a). Interestingly when the activity of SOD decreased at 18:00 h, CAT activity increased at the same time point.

3.1.4. Total antioxidant status of the tissue against ABTS radical cation

Total antioxidant status (TAS) for 24 h duration at an interval of 4 h was measured. A significant variation in percentage inhibition/

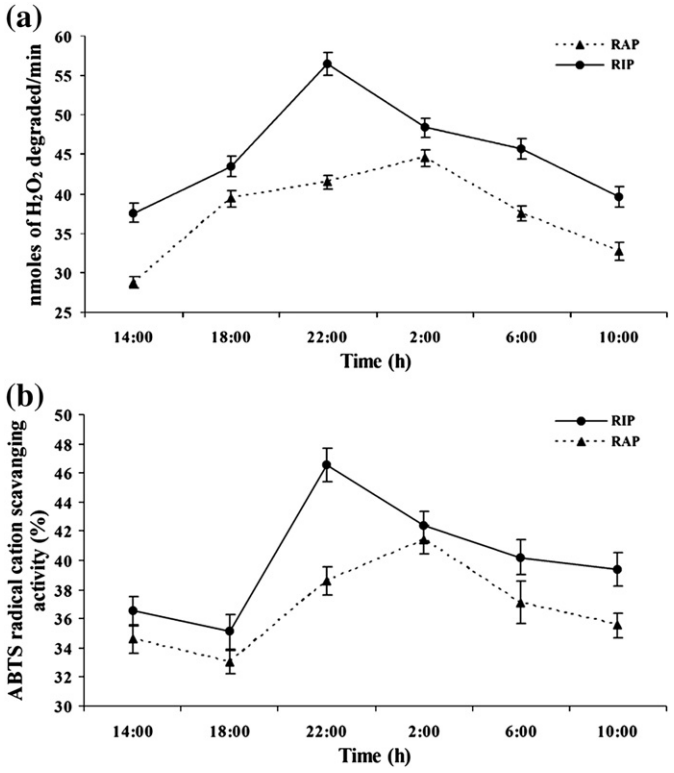

Fig. 2. (a) 24 h rhythm of catalase activity in the lung of birds in groups at different time points (14:00, 18:00, 22:00,2:00, 6:00 and 10:00) during reproductively active (RAP; June) and inactive phase (RIP; December). (b) Time course of ABTS radical cation scavenging activity (indicated as %) in the lung of birds in groups at different time points (14:00, 18:00, 22:00,2:00, 6:00 and 10:00) during reproductively active (RAP; June) and inactive phase (RIP; December). Complete decolorization of the ABTS radical cation was accepted as 100%.Data are expressed as the Mean ± SEM, $n = 5$ for each group.

elimination of ABTS radical cation was noted in the lung tissue of the bird. TAS in terms of ABTS radical cation scavenging activity was found to be maximum at 2:00 h during RAP and 22:00 h during RIP and minimum at 18:00 h during RAP as well as RIP. TAS showed gradual increase after 18:00 h reaching to maximum at 2:00 h in RAP and 22:00 h in RIP and again declined to 18:00 h in both reproductive phases (Fig. 2b).

3.1.5. Western blot analysis

In order to determine a relationship between circulatory melatonin level and the expression of RORα in lung, we compared the 24 h profiles of both the circulatory level of melatonin and RORα expression in lungs. There was gradual variation in expression of RORα receptor during 24 h in both reproductive phases. Minimum expression of RORα receptor was found at 22:00 h and 02:00 h during reproductively inactive (RIP) and active phase (RAP) respectively and maximum at 18:00 (Fig. 3).

3.2. Effect of melatonin injection

3.2.1. Melatonin level

The melatonin treated group of birds showed a significant ($P<0.01$) increase in circulatory level of melatonin than control birds receiving saline during the RAP. A similar pattern in circulatory melatonin level was seen for the melatonin treated group of birds during RIP. The groups (control + melatonin treated) of birds during RIP showed significantly ($P<0.01$) higher level of peripheral melatonin than groups (control + melatonin treated) of birds during RAP (Fig. 4a).

3.2.2. Lipid peroxidation (LPO) assay by TBARS level estimation

The melatonin treated group of birds showed a significant ($P<0.01$) decrease in malondialdehyde level than control birds receiving saline during the RAP. A similar pattern in malondialdehyde level was seen for the melatonin treated group of birds during RIP. The groups (control + melatonin treated) of birds during RIP showed significantly ($P<0.01$) lower level of malondialdehyde than groups (control + melatonin treated) of birds during RAP (Fig. 4b).

3.2.3. Antioxidative enzymes activity

The melatonin treated group of birds showed a significantly ($P<0.01$) higher activity of both SOD and catalase than control birds

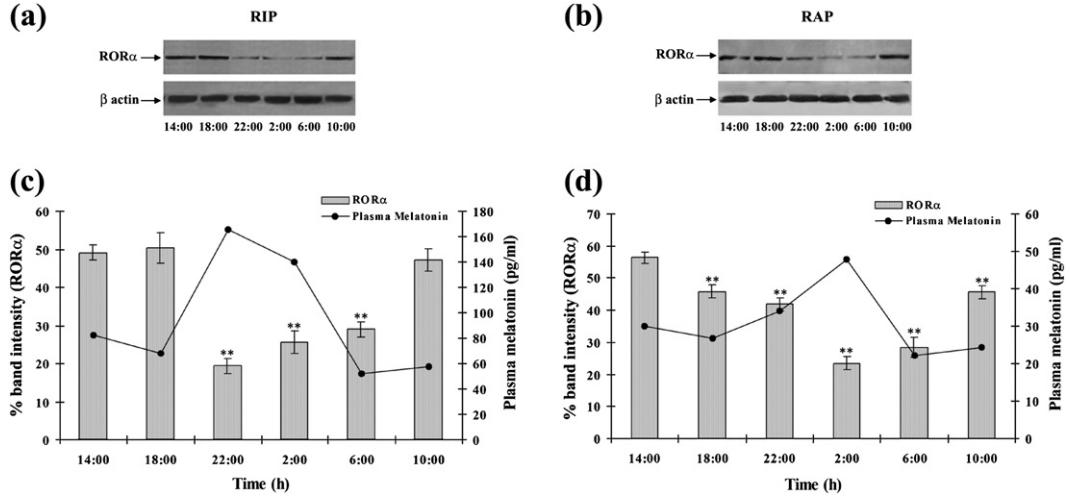

Fig. 3. (a) Western blot showing variation in expression of RORα receptor in isolated lung protein from the lung of *Perdicula asiatica* at different time points (14:00, 18:00, 22:00,2:00, 6:00 and 10:00) during reproductively inactive phase (RIP).(b) Histogram showing percent band intensity of RORα receptor in isolated lung protein of *Perdicula asiatica* at different time points (14:00, 18:00, 22:00, 2:00, 6:00 and 10:00) during reproductively inactive phase (RIP). (c) Western blot showing variation in expression of RORα receptor in isolated lung protein from the lung of *Perdicula asiatica* at different time points (14:00, 18:00, 22:00,2:00, 6:00 and 10:00) during reproductively active phase (RAP).(d) Histogram showing percent band intensity of RORα receptor in isolated lung protein of *Perdicula asiatica* at different time points (14:00, 18:00, 22:00,2:00, 6:00 and 10:00) during reproductively active phase (RAP). % band intensity is an arbitrary unit used to show the comparative expression of receptors. It was calculated as; band intensity of RORα divided by band intensity of β-actin and then multiplying by 100. Data are expressed as the Mean ± SEM, $n = 5$ for each group.

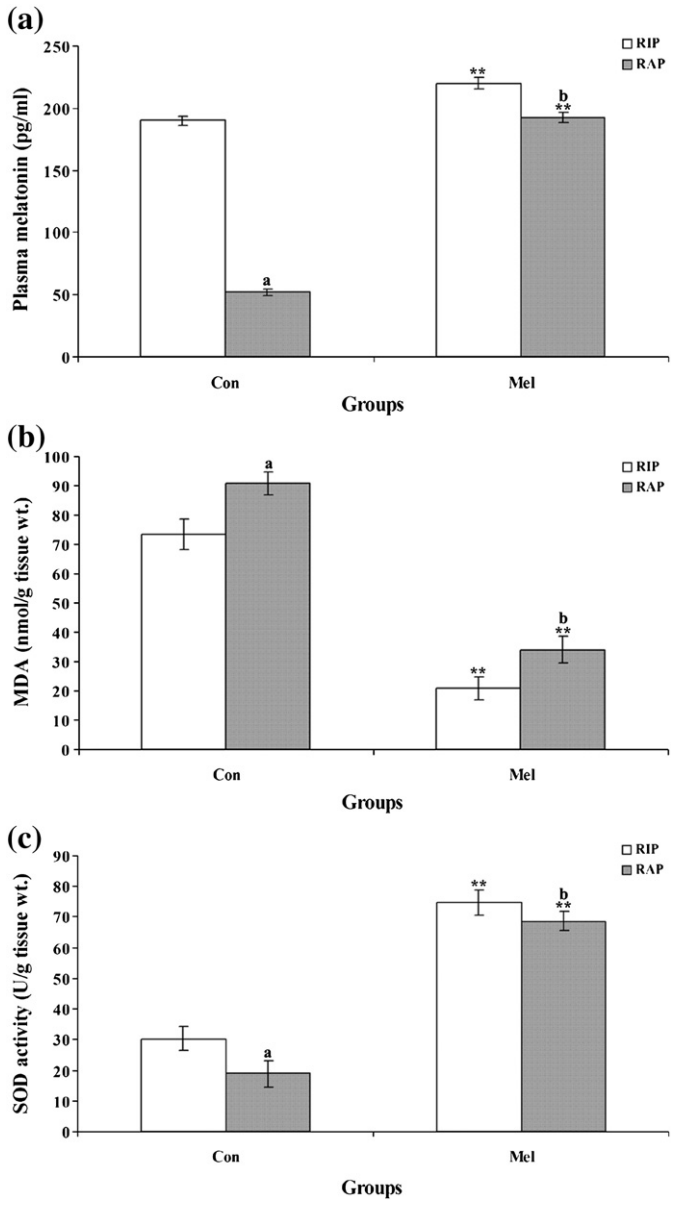

Fig. 4. Effect of melatonin injection on: (a) plasma melatonin concentrations, (b) lipid peroxidation and (c) superoxide dismutase activity in lung of *Perdicula asiatica* during two reproductive phases (RAP and RIP).Data are expressed as the Mean ± SEM, $n = 5$ for each group.

receiving saline during the RAP. A similar pattern in SOD and catalase activity was seen for the melatonin treated group of birds during RIP. The groups (control + melatonin treated) of birds during RIP showed significantly ($P<0.01$) higher enzymes (SOD and catalase) activity than groups (control + melatonin treated) of birds during RAP (Figs. 4c,5a).

3.2.4. Total antioxidant status of the tissue against ABTS radical cation

The melatonin treated group of birds showed a significantly ($P<0.01$) higher percentage of inhibition of ABTS radical cation than control birds receiving saline during the RAP. A similar pattern in percentage of inhibition of ABTS radical cation was seen for the melatonin treated group of birds during RIP. The groups (control + melatonin treated) of birds during RIP showed significantly ($P<0.01$) higher percentage of inhibition of ABTS radical cation than groups (control + melatonin treated) of birds during RAP (Fig. 5b).

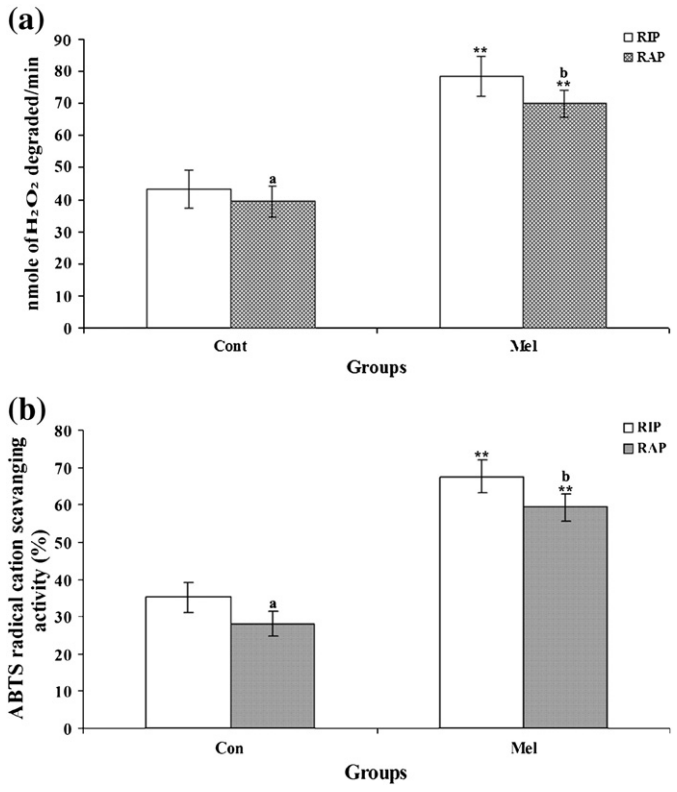

Fig. 5. Effect of melatonin injection on: (a) catalse activity and (b) total antioxidant status in lung of *Perdicula asiatica* during two reproductive phases (RAP and RIP). Complete decolorization of the ABTS radical cation was accepted as 100%. Data are expressed as the Mean ± SEM, $n = 5$ for each group.

3.2.5. Western blot analysis

Expression of RORα receptor in melatonin treated birds decreased as compared to control group birds during RIP as well as during RAP, but the receptor expression was more during RAP as compared to RIP (Fig. 6).

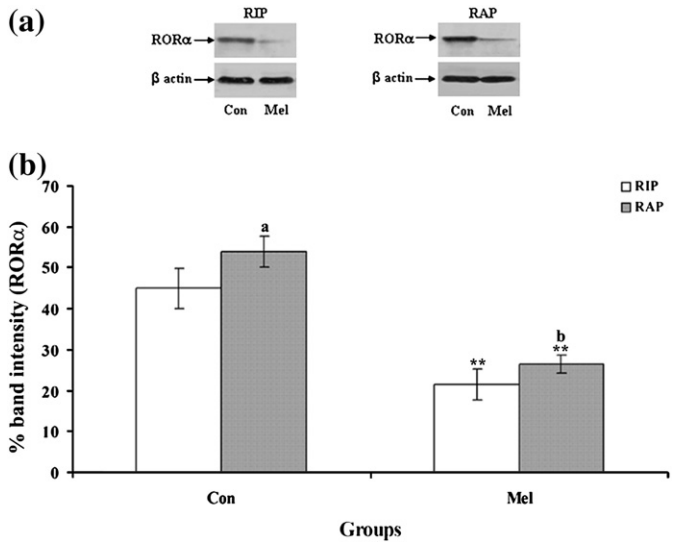

Fig. 6. (a) Western blot showing effect of melatonin on expression of RORα receptor in isolated lung protein from lung of *Perdicula asiatica.* (b) Histogram showing percent band intensity of RORα receptor in control and melatonin treated birds during both reproductive phases (RIP and RAP). % band intensity is an arbitrary unit used to show the comparative expression of receptors. It was calculated as: band intensity of RORα divided by band intensity of β-actin and then multiplying by 100. Data are expressed as the Mean ± SEM, $n = 5$ for each group.

4. Discussion

Melatonin participates in various lines of oxidative defense and countless publications have dealt with protective action of melatonin. Till date no report exists suggesting the oxidative stress defense mechanism in avian lung and role of melatonin. Antioxidant actions of melatonin were strongly stimulated by the finding of potent direct scavenging of hydroxyl radicals by this endolamine (Tan et al., 1993). At a pharmacological concentration the value of melatonin as a direct scavenger have been repeatedly demonstrated (Hardeland, 2008).

In order to establish that avian lung could also be a target for free radical load we first present the result of daily variation of the free radicals during two reproductive phases i.e. active and inactive and compared the same with the circulatory level of melatonin to establish it as an internal free radical scavenger. On the other hand, to support our above hypothesis we also noted the effect of melatonin injection (evening hours) during two reproductive phases to delineate whether circulatory melatonin level have any role in regulating the free radical load during reproductively active and inactive phase when its peripheral level is low and high respectively.

In the result of the first set of experiment about daily variation we found a definite and direct correlation of SOD, catalase and ABTS cation with high level of melatonin during night time (i.e. 18:00 h onwards till 2:00 h). Contrary to this being independent of reproductive phases an inverse relation of MDA was observed with the level of melatonin. It has been published that daily circulatory level of melatonin present a drastic difference in the basal and peak value during both the reproductive phases i.e. high during RIP and low during RAP (Kharwar and Haldar, 2011c). In the second set of experiment we wanted to observe whether low circulatory level of melatonin during RAP, if induced by exogenous melatonin injection can reduce the oxidative stress of the lung tissue to the level of reproductively inactive phase. As per hypothesis exogenous melatonin administration during RAP reduced significantly the MDA level and increased the SOD, catalase and ABTS cation scavenging capacity to the level of RIP indicating clearly that exogenous melatonin can prevent the oxidative damage of different tissue (as here it is lung) under oxidative stress.

On the other hand injection of melatonin during RIP (which also increased the basal level of melatonin during RIP) was able to reduce significantly the level of MDA and increased the activity of SOD and catalase and ABTS cation scavanging capacity more than the control (saline injected) birds. The above effect was quite similar to the results obtained from the birds having injection of melatonin during RAP supporting once again the hypothesis that melatonin is a free radical scavenger and regulates the daily variation of free radicals during different reproductive phases which is of great adaptive significance for the wild birds.

The genomic action of melatonin has been reported via the orphan receptors RORs. Recent studies of ROR are greatly widened our understanding of the physiological role of this nuclear receptor subfamily and provided exciting clues about their critical functions such as detoxification, immunity, cellular metabolism and circadian rhythm etc. We thought to extend our study by estimating the expression of RORα, a member of a subfamily of nuclear receptor in above set of experiments.

The daily variation of RORα expression in lung of *P. asiatica* provided a reproductive phase dependent variation being inversely related with circulatory melatonin level i.e. when melatonin is high expression of RORα is drastically reduced. As we know that the light regulates the level and duration of melatonin, hence the duration of reduced RORα was more during RAP than RIP suggesting that melatonin might be acting via RORα expression.

Our suggestion got further support from experiment on melatonin injection which reduced RORα expression being irrespective of reproductive phases. Several studies have revealed a connection between RORα expressions being implicated in stress related regulatory functions. Hence, our data suggest that avian lung is susceptible for free radical load or oxidative stress. Circulatory level of melatonin in this regard acts as a scavenger via RORα and thereby protects the birds.

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