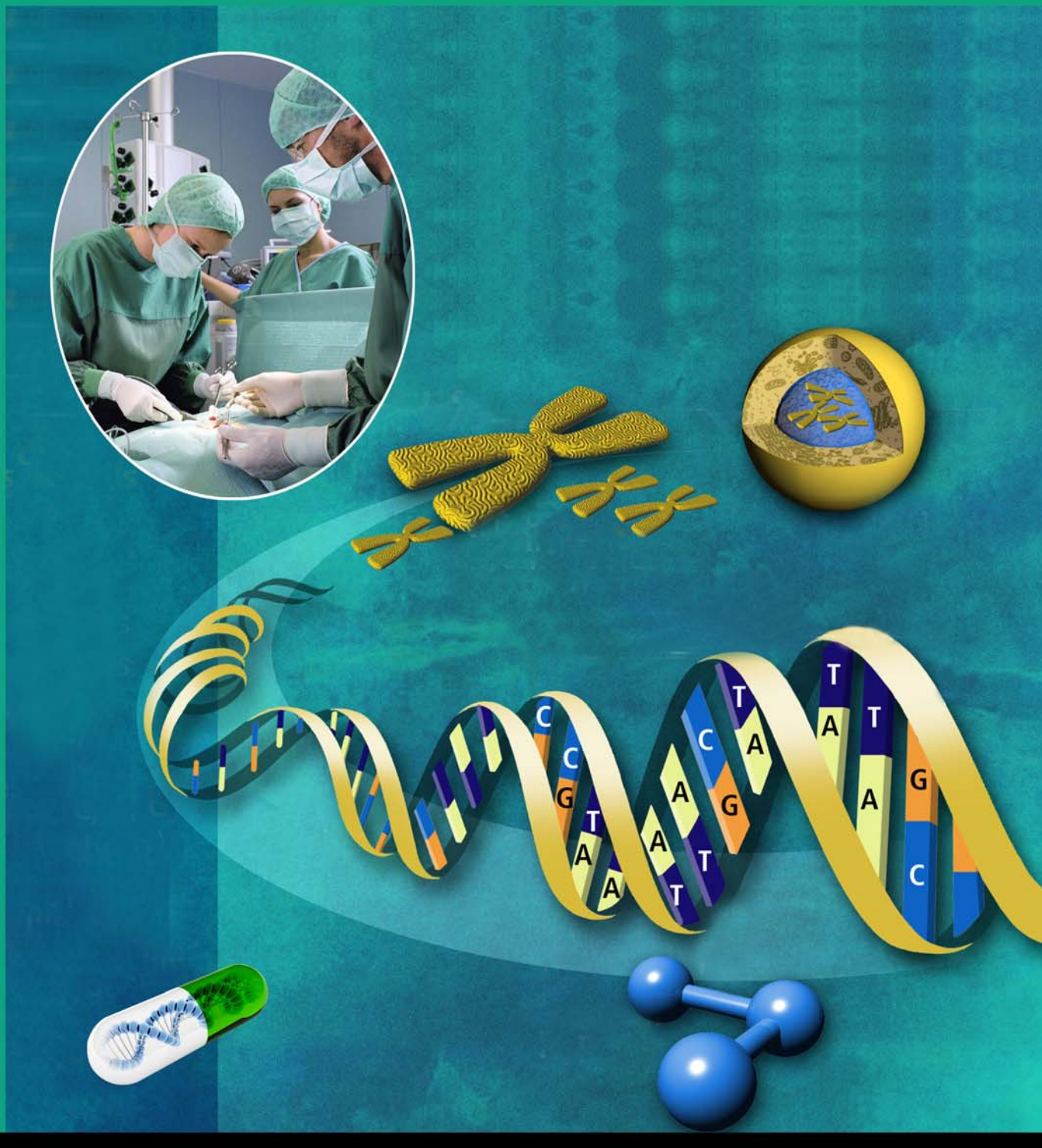
ISSN 2278 - 5221
Vol. 1, No. 2, October 2012

International Journal of Pharma Medicine and Biological Sciences

IJPMBS



WWW.IJPMBS.COM

editorijpmbs@gmail.com or editor@ijpmbs.com

International Journal of Pharma Medicine and Biological Sciences

ISSN 2278 – 5221 www.ijpmbs.com
Vol. 1, No. 2, October 2012

Research Paper

EFFECT OF PHOTOPERIOD ON NITRITE PRODUCTION BY LEUCOCYTES IN FRESH WATER SNAKE, *NATRIX PISCATOR*

Alka Singh[1] and Ramesh Singh[1*]

Corresponding Author:* ***Ramesh Singh,* ✉ *upcrsingh@yahoo.com*

Recent evidence suggests that immune function varies substantially on seasonal basis. The primary cue to perceive seasonal change is change in photoperiod. The purpose of the present experiment was to study the role of photoperiodic manipulation on the nitric oxide production by peripheral blood leucocytes in the fresh-water snake, *Natrix piscator*. To study effect of photoperiod, animals were subjected to 24 hour continuous light and continuous dark for 30 days. Animals kept in natural day length served as control. At termination of experiments, animals were sacrificed, and blood was collected. Leucocytes were isolated and were incubated for 24 hours and nitric oxide production was measured by measuring the nitrite concentration. Nitrite production was significantly decreased to the cultures obtained from the animals kept under continuous light while nitrite concentration was increased in animals kept under continuous dark, when compared with the animals kept under natural day length. The possible role of increased melatonin synthesis in dark was suggested to increase the nitric oxide production.

Keywords: Nitric oxide, Photoperiodic manipulation, Snake, Leucocytes

INTRODUCTION

Seasonal phenomena may be imposed on animals by their environment and reflect an interaction between an individuals intrinsic seasonal clocks and the environment. Most formal studies on seasonality have focused on day lengths, i.e., photoperiod as the environmental cue used by animals to coordinate intrinsic seasonal rhythms with extrinsic seasonal environmental changes. Animals undergo seasonal changes in physiological state. Seasonal changes in photoperiod act as direct cues to predict the time of the year. Photoperiodic information is used to initiate or terminate specific seasonal adaptations (Nelson and Demas, 1997). Most studies on seasonality have focused on the role of photoperiod in providing temporal information for reproduction (Reiter, 1991). Environmental factors, such as day length, food availability, temperature, and social interaction, can have pronounced effects on immune function (Klein and Nelson, 1999). Peripheral blood leucocytes

[1] Department of Zoology, Udai Pratap Autonomous College, Varanasi -221002, Uttar Pradesh, India.

are primary immune cells and important elements of non specific immunity.

Effect of photoperiodic manipulation on immune parameters have been documented in non tropical rodent species including deer mice (*Peromyscus maniculatus*) (Champney and McMurray, 1991) and prairie voles (*Microtus ochrogaster*) (Nelson *et al.,* 1996), as well as Syrian (*Mesocricetus auratus*) (Brainard *et al.,* 1988) and Siberian (*Phodopus sungorus*) (Drazen *et al.,* 2002; Yellen *et al.,* 1999) hamsters. In some species (e.g. Siberian hamsters, prairie voles) specific immune responses are suppressed in short days. In contrast, individuals of other species (e.g. deer mice, Syrian hamsters) display enhanced immune functions in short winter-like day lengths compared with long summer-like days. Some of the earliest experimental studies implicating melatonin in changes in immune functions involved the functional suppression of melatonin concentration via experimental manipulation of the photoperiod. Effect of photoperiodic manipulation on immune parameters have also been documented in siberian hamsters (Bilbo and Nelson, 2004; Demas *et al.,* 2003).

Nitric Oxide (NO) produced endogenously from L-Arginine by nitric oxide synthetases. Nitric oxide is extremely unstable. It undergoes rapid oxidative degradation to nitrite (NO_2^-) and nitrate (NO_3^-), which can be spectrophotometrically determined. NO plays an important role in many physiological processes including vascular regulation, immune responses, and neural communication. Some reports are available on seasonal variation in cell-mediated innate immune responses in reptiles (Garcia and Fuente, 1991; Zapata *et al.,* 1992; Munoz *et al.,* 2000; Munoz and Fuente, 2001). Mondal and Rai (2000) have studied the effect of temperature on nitrite production by splenic macrophages in wall lizard, *Hemidactylus flaviviridis*. Study regarding photoperiodic manipulation and nitric oxide production is lacking in fresh-water snake. Hence, present study was undertaken to study the effect of photoperiodic manipulation on nitric oxide production by leucocytes in this species.

MATERIALS AND METHODS

Animals

Male fresh-water snakes, weighing 80-120g, were obtained from a local supplier who collected these animals in the suburbs of Varanasi (28^0 18'N; 83^0 1'E). Animals were brought to the unconditioned laboratory. Animals were housed in vivarium (wood and wire net cages; size 50 x 30 x 30 cm). Each cage had an earthen bowl (4L capacity) filled with water and accommodated 4-5 snakes. Snakes were fed on small fishes once a week. Cages were cleaned, and bowl water was changed next day following feeding. The guidelines of the committee for the purpose of control and supervision of experiment on animals (CPCSEA), Ministry of Statistics and Programme Implemen-tation, Government of India, were followed in maintenance and sacrifice of animals.

Chemicals

Culture medium (RPMI-1640), L-glutamine, gentamycin, fetal bovine serum (FBS), and other chemicals were purchased from Himedia Laboratories Pvt. Ltd. (India). The culture medium was supplemented with 1 µl ml^{-1} gentamycin, 10 µl ml^{-1} of 200 mM L-glutamine, 10 µl ml^{-1} anti-anti (Gibco) and 5% FBS and referred to as complete culture medium.

Experiment

Animals were divided into three groups of five

animals each: Group one animals were maintained in natural light dark cycle (10L:14D) and served as control, group two animals, in continuous light (24L), and group three animals, in continuous dark (24D) for 30 days. Animals were sacrificed at the termination of experiment and blood was drawn in heparinized tubes through cardiac puncture and stored at 4°C.

Total Leucocyte Count (TLC)

TLC was performed using Neubauer chamber and routine hematological methods.

Isolation of Peripheral Blood Leucocyte

Peripheral Blood Leucocytes (PBL) were collected from the buffy coat (the layer of PBLs between the plasma and RBCs) using a slow spin technique as described by Keller *et al.* (2005). The tubes were centrifuged at 42 x g for 25 min at 8°C. The PBLs were collected by gently swirling the buffy coat into the plasma and transferring the cells into a new tube. Following centrifugation at 200 x g for 10 min, the plasma was removed and the cell pellet was gently resuspended in 1 ml of complete culture medium. Leucocytes were counted and adjusted to $1x10^6$ cells ml^{-1} in complete RPMI. Cell viability was checked through trypan blue exclusion test, which exceeded 95%.

Nitrite Assay

Nitrite assay was performed following the method of Ding *et al.* (1988). In brief, 100 µl of leucocytes (10^5 cells) or standard $NaNO_3$ was added to 96 well, culture plate in triplicate and incubated for 24 hours at 25 °C. Plate was then centrifuged at 200 x g for 10 minutes. 50µl of supernatant and 50 µl of Griess Reagent (25 µl of 1% sulphanilamide prepared in 3 N HCl and 25 µl of 0.1% N-naphthyl ethylenediamine prepared in distilled water) was mixed in microplate and after 10 minutes, absorbance was measured at 540 nm with the help of ELISA plate reader (Thermo). Different concentrations of sodium nitrite were used to obtain the standard curve.

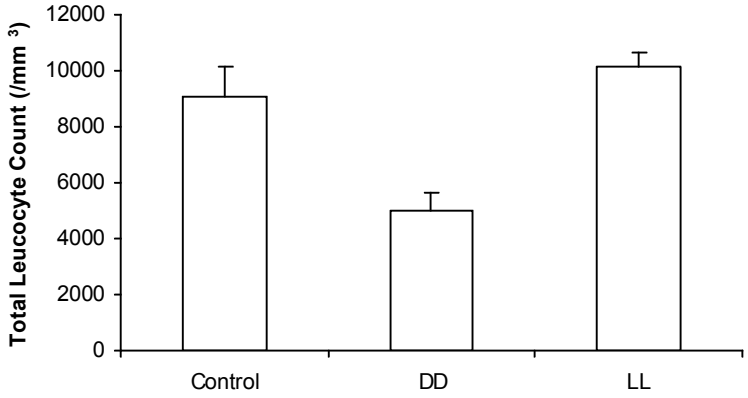
Nitrite concentration (µM) was calculated for each sample. Data are presented as mean ± SEM. Means were compared, and statistical difference between means was determined by Student's t-test.

RESULTS AND DISCUSSION

Total leucocyte count was significantly decreased in animals kept under continuous dark when compared to control (Figure 1). Nitric oxide production, as measured by nitrite concentration, was significantly decreased ($p < 0.05$) to the cultures obtained from the animals kept under continuous light. In contrast there was significant enhancement in nitric oxide production by leucocytes obtained from animals kept under continuous dark, when compared with animals kept under natural day length (Figure 2).

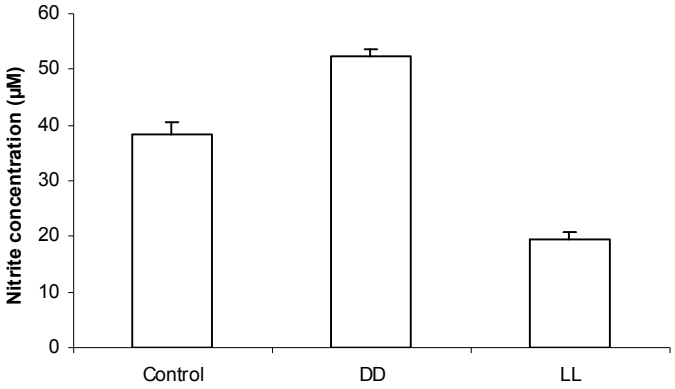
In the present study, leucocyte number was significantly decreased in the snakes kept under continuous dark. This is in contrast to that reported by Bilbo *et al.* (2003) and Prendergast *et al.* (2002); they have reported increased total leucocyte number in short day mammalian species. This may be due to species specific differences. In birds, Kliger *et al.* (2000) have reported increased heterophil number but decreased lymphocytes in chickens exposed to continuous light. Reactive nitrogen intermediates (RNI), produced by leucocytes and other cell types in response to IFN-α, or IFN-α plus tumour necrosis factor-a (TNF-a) and interleukin-1 (IL-1), have been shown to play an important role in killing of pathogens (Flesch and Kaufmann, 1991). In the present study nitric oxide production

Figure 1: Effect of Photoperiod on Total Leucocyte Count in the Fresh-Water Snake, *Natrix piscator*



Note: DD – Complete dark; LL – Complete light, * $p < 0.05$

Figure 2: Effect of Photoperiod on Nitric Oxide Production by Peripheral Blood Leucocytes in the Fresh-Water Snake, *Natrix piscator*



Note: DD – Complete dark; LL – Complete light, * $p < 0.05$

was significantly reduced in the animals kept under continuous light which might be result of reduced synthesis of melatonin in light – i.e., physiological pinealectomy. In analyzing the influence exerted by the light regimen upon innate immune functions of blood leucocyte, two distinct aspects have to be considered: the circadian structure of the rhythms and the level of the assessed functions. Mondal and Rai (2000), have shown that lower temperature suppresses macrophage phagocytosis and nitrite release by splenic macrophages in wall lizard. Sex steroids also play an important role in suppression of nitrite release. There are several indirect and also direct indicators that melatonin, secreted exclusively at night, would play a role in the immune function. *In vitro* studies employing pharmacological doses of melatonin (5-100 µM) revealed a dose-dependent activation of phagocytic function (Rodriguez *et al.,* 1999).

However, such doses are far above the physiologically available range. In summary, the result of the present study shows that continuous light exposure to fresh-water snake reduces nitric oxide production, while continuous dark increases nitric oxide by peripheral blood leucocytes.

CONCLUSION

This study is helpful in understanding the comparative immunology of the reptiles, a phylogenetically important group being ectothermic amniotes. The present work, for the first time, elucidates the role of photoperiod on nitric oxide production in *N. piscator*. In brief, findings of this study indicates that continuous dark enhances innate immune function in fresh-water snake.

ACKNOWLEDGMENT

Financial Grant (SR/ SO/ AS–15/ 2005) from DST, New Delhi, Govt. of India to RS is gratefully acknowledged.

REFERENCES

1. Bilbo S D, Dhabhar F S, Vishwanathan K, Saul A and Nelson R J (2003), "Photoperiod affects the expression of sex and species differences in leucocyte number and leucocyte trafficking in congeneric hamsters", *J. Psychoneuroendocrinol.,* Vol. 28, pp. 1027-1043.
2. Bilbo S D and Nelson R J (2004), "Photoperiod influences the effects of exercise and food restriction on an antigen specific immune response in Siberian hamsters", *Endocrino.,* Vol. 145, No. 2, pp. 556-564.
3. Brainard G C, Watson-Whitmeyer M, Knobler R L and Lubin F D (1988), "Neuroendocrine regulation of immune parameters: photoperiod control of spleen in the Syrian Hamsters", *Ann. N .Y. Acad. Sci.*, Vol. 540, pp. 704-706.
4. Champney T H and McMurray D N (1991), "Spleen morphology and lymphoproliferative activity in short photoperiod exposed hamsters", in *Role of Melatonin and Pineal Peptides in Neuroimmunomodulation,* Fraschini F and Reiter R J (Eds.) Plenum Press, London, pp. 219-225.
5. Demas G E, Bartness T J, Nelson R J and Drazen D L (2003), "Photoperiod modulates the effects of norepinephrine on lymphocyte proliferation in Siberian hamsters", *Am. J. Physiol. Regul. Integr. Comp. Physiol.,* Vol. 285, pp. R873-R879.
6. Ding A H, Nathan C F and Stuehr D (1980), "Release of reactive nitrogen intermediates and reactive oxygen intermediates from mouse peritoneal macrophages. Comparison of activating cytokines and evidence for independent production", *J. Immunol.,* Vol. 141, pp. 2407-2412.
7. Drazen D L, Jasnow A M, Nelson R J and Demas G E (2002), "Exposure to short days, but not short term melatonin, enhances humoral immunity of male Syrian hamsters (*Mesocricetus auratus*)", *J. Pineal Res.,* Vol. 33, pp. 118-124.
8. Flesch I E A and Kaufmann S H E (1991), "Mechanisms involved in mycobacterial growth inhibition by gamma interferon-activated bone marrow macrophages: role of reactive nitrogen intermediates", *Infect. Immunol*., Vol. 59, pp. 3213-3218.
9. Garcia S and De la Fuente M (1991),

"Seasonal variations in the activity of *in vitro* peripheral blood granulocytes in the turtle *Mauremys caspica", Comp. Biochem. Physiol*., Vol. 100, pp. 697-702.

10. Keller J M, McClellan-Green P D, Lee A M, Arendt M D, Maire P P, Segars A L, Whitaker J D, Keil D E and Peden-Adams M M (2005), "Mitogen-induced lymphocyte proliferation in loggerhead sea turtles: comparison of methods and effects of gender, plasma testosterone concentration, and body condition on immunity", *Vet. Immunol. Immunop*., Vol. 103, pp. 269-281.

11. Klein S L and Nelson R J (1999), "Influence of social factor on immune function and reproduction" *Rev. Reprod.,* Vol. 4, pp.168-178.

12. Kliger C A, Gehad A F, Hulet R M, Roush W B, Lillehoj H S and Mashaly M M (2000), "Effect of photoperiod and melatonin on lymphocyte activities in male broiler chicken", *Poul. Sci.,* Vol. 79, pp. 18-25.

13. Munoz F Z, Galvan A, Lerma M and De la Fuente M (2000), "Seasonal changes in peripheral blood leucocyte functions of the turtle *Mauremys caspica* and their relationship with corticosterone, 17- beta-estradiol and testosterone serum levels", *Vet. Immunol. Immunop*., Vol. 77, pp. 27-42.

14. Munoz F Z and Fuente M (2001), "The immune response of thymic cells from the turtle *Mauremys caspica*", *J. Comp. Physiol. B*, Vol. 171, pp. 195-200.

15. Nelson R J, Fine J B, Demas G E and Moffatt C A (1996), "Photoperiod and population density interact to affect reproductive and immune function in male prairie voles", *Am. J. Physiol.* Vol. 270, pp. 571-577.

16. Nelson R J and Demas G E (1997), "Role of melatonin in mediating seasonal energetic and immunologic adaptations", *Brain Res. Bull.,* Vol. 44, pp. 423-430.

17. Prendergast B J, Edward K E W, Yellen S M and Nelson R J (2002), "Photorefractoriness of immune function in male Siberian hamsters (*Phodopus sungorus*)", *J. Neuroendocrinol.,* Vol. 14, pp. 318-329.

18. Reiter R J (1991), "Pineal melatonin, cell biology of its synthesis and of its physiological interactions", *Endocrine Rev.,* Vol. 12, pp. 151-180.

19. Rodriguez A B, Marchena J M, Nogales G, Duram J and Barriga C (1999), "Correlation between the circadian rhythm of melatonin, phagocytosis, and superoxide anion levels in ring dove heterophils", *J. Pineal Res.* Vol. 26, pp. 35-42.

20. Mondal S and Rai U (2000), "In vitro effect of temperature on phagocytic and cytotoxic activity of splenic phagocytes of the wall lizard, *Hemidactylus flaviviridis*", *Comp. Biochem. Physiol. A*, Vol. 129, pp. 391-398.

21. Yellon S M, Fagoga O R and Cannarella S L N (1999), "Influence of Photoperiod on immune cell functions in the male Siberian hamsters", *Am. J. Physiol. Regul. Integr. Comp. Physiol*., Vol. 276, pp. R97-R102.

22. Zapata A G, Varas A and Torroba M (1992), "Seasonal variations in the immune system of lower vertebrates", *Immunol. Today*, Vol. 13, pp. 142-147.

International Journal of Pharma Medicine and Biological Sciences
Hyderabad, INDIA. Ph: +91-09441351700, 09059645577
E-mail: editorijpmbs@gmail.com or editor@ijpmbs.com
Website: www.ijpmbs.com

ISSN 2278-5221
02
9 772278 522003