"Gene expression studies on mechanisms underlying the role of melatonin hormone as free radical scavenger and developmental enhancer in insects"

Ref. No: 42-201/2013 (SR), Dt; 22-03-2013

In the present study the effect of melatonin supplementation on antioxidant and insect development was studied. Melatonin is a hormone secreted by Pineal gland and extra pineal tissues. The results show that chronic dosage of melatonin results in increased antioxidant levels thereby reducing ROS. Long-term melatonin supplementation led to increased survival rate and reversed the detoxification enzyme levels in pesticide exposed larvae, suggesting an interaction between melatonin and larval insect antioxidant defense system. This suggests that melatonin is involved in free radicals scavenging, thereby is involved in protecting the insect from the toxic effects of pesticide exposure. Melatonin in midgut of *S. litura* modulates the ADS to cope with oxidative stress in a time dependent manner, showing melatonin presence in extra pineal tissues. Melatonin also is effective as antioxidant, as evidenced by its protective role in insect midgut in response to pesticide exposure SOD and CAT levels were increased with decreased TBARS levels.Midgut also shows circadian rhythmcity in digestive enzyme secretion which is confirmed by our studies on carbohydrases and proteases. Our study also confirms that the circadian rhythm and peripheral head clock expression with reference to cry1 and cry2 gene expression. Hox genes are involved in the insect development. The physiological mechanisms of the photoperiod underlying the developmental genes remain little understood. The developmental gene expression such as Dfd, Lab, abd-A, Abd-B, Ubx, melatonin (AA-NAT) and reproductive proteins like Vg was analyzed in *S. litura* at different photoperiod regimens. Deformed and Labial mRNA levels do change in response to 24L: 0D photoperiod. Abd-A, Abd-B and AA-NAT gene expression was higher at MEL+ABA group of 0L: 24D as compared with other photoperiods. The effect of melatonin and photoperiod are involved in the insect development. Insects use the photoperiod as a temporal cue to initiate developmental events such as molting, eclosion and diapause (Zaslavski, 1988). In *S.litura*, melatonin supplementation significantly increased the NAT activity in 0L: 24D which suggests that melatonin secretion in insects occur during dark period like in case of higher animals. This is substantiated by HPLC chromatogram which shows a higher melatonin peak in 0L: 24D than 24L:0D photoperiod. In conclusion, the present study is the first report of Melatonin presence in head and hemolymph in *Spodoptera litura*. Exogenous melatonin has a protective role in insects by activating the antioxidant defense

system in response to toxic insult. Melatonin secretion and aa-NAT activity peaks during prolonged darkness suggesting that like vertebrates melatonin is produced in darkness. Melatonin interaction with circadian clock genes and vitellogenin gene is established from this study.

Achievements from the Project

- This is the first report of melatonin presence in head and hemolymph in insects.
- Exogenous Melatonin has a protective role in insects by activating the antioxidant system in response to toxic insult.
- Melatonin secretion and aa-NAT activity peaks during prolonged darkness suggesting that like vertebrates melatonin is produced in dark.
- Melatonin interaction with circadian clock genes and vitellogenin gene is established from this study

Contribution to the Society

Melatonin is a hormone which is produced by pineal and extra pineal tissues in organisms. The results of the present study show that melatonin is secreted during darkness. Melatonin is also known to be a potent antioxidant which can reverse the toxic effects of pesticides in insects. Based on the above since insect control require the use of chemical insecticides, the time and quantity of spray will differ based on the photoperiod to get the optimum results. Further this study also shows that besides being involved in reproduction in higher animals, melatonin also functions as an antioxidant in insects. The results of the present study can be useful in regulating the timing of the insecticide treatment, so that the use of chemical insecticides can be reduced by appropriate timing of insect control.

Conclusion

- Long-term melatonin exposure, leads to increased survival rate in lepidopteran insect larvae and also resulted in significant reduction in the detoxification enzyme levels; This suggest the role of melatonin in scavenging the toxic free radicals and also modulates the antioxidant defense system of *S. litura* midgut to cope with oxidative stress in a time dependent manner.

- In circadian clock gene expression study, we extended the knowledge of adult heads circadian rhythms to a new insect order, lepidoptera, by demonstrating in the moth *S. litura* the presence of a peripheral heads clock along with a circadian rhythm in period, cry1 and cry2 gene expression. Also, it will be necessary to study the relative autonomy of the adult heads clock, since no paradigm has emerged from insect studies on the distribution and functional organization of circadian clocks.
- Although Hox genes play a major role in shaping the anterior-posterior body axis during animal development, our understanding of how they act in different photoperiods at precise larval developmental stages is rudimentary. The physiological mechanisms of the photoperiod underlying the developmental genes remain little understood. Deformed and Labial mRNA levels did change in response to dark photoperiod. It may be possible the Hox genes (Dfd, Lab) controls the insect head development by the induction of the melatonin synthesis through photoperiod. Abd-A, Abd-B and AA-NAT gene expression was higher at dark photoperiod than other photoperiods, suggesting melatonin secretion during dark period may influence the abdominal and wing development control
- The midgut digestive system of *S. litura* is as diverse as that of any of other polyphagous lepidopteran insect species. This study used to evaluate the response of photoperiod as digestive enzyme targets of insects particularly in carbohydrases and proteases. Characterization of the digestive enzymes of *S. litura* offers an opportunity to control the insect growth by the variation of dark/light conditions. This study also shows that circadian rhythm is influenced the digestive enzymes some extent by the changes in the light/dark period. Further studies on gene expression mechanisms of the circadian clock required to improve our understanding of the mechanisms underlying the release of digestive enzymes controlled by the photoperiod.
- In the histological studies, we made an attempt to elucidate that melatonin and luzindole or photoperiod has a significant damage in insect midgut and abdomen of the insect. *S. litura* midgut was similar to those described in the previous literatures in Lepidoptera. However, the bifurcated microvilli suggest the ultrastructural modifications due to the luzindole in the midgut of nocturnal insect exposed to LL and DD conditions. The luzindole combined with the photoperiod make the histological changes of the insect midgut, suggesting that *S. litura* may be susceptible to the timing exposure of the melatonin hormone agonist,

luzindole. But this hypothesis needs further investigation of the mechanism of time-dependent action of the luzindole in the pesticide formulations.

- In insect development studies, the presence of melatonin in the insect head is involved in the phenomenon of photoperiodism than the hemolymph of insects. In addition, insects use the photoperiod as a temporal cue to initiate developmental events such as molting, eclosion and diapause. Therefore, melatonin in the nocturnal insect head may be a neurochemical mediator of photoperiodic control of developmental events.

Recommendations

- This study deals with understanding the role of melatonin hormone on the developmental fitness and insecticide detoxifying potential of lepidopteran insects. The results of the present study can be useful in regulating the timing of the insecticide treatment, so that the use of chemical insecticides can be reduced by appropriate timing of insect control strategies.
- The control of the development of insects is achieved via melatonin hormone using photoperiod i.e., day/night variations. Understanding the mechanism of the insect developmental biology through the melatonin hormone biosynthesis pathway helps to control the development of various agricultural pests using light/dark conditions.
- This new concern in the area of the developmental biology is cost-effective and not causing any impact on other Non-target organisms. This study used to understand the concept of ageing through antioxidant pathway and developmental patterns in animals.
- The study will help in finding out the possible interaction between Hox proteins and melatonin which regulate the timing of insect development. Understanding their interaction helps in updating our information about developmental patterns in humans.
- This is the first report of invertebrates particularly in insects regarding the melatonin role as indirect antioxidant, photoperiodic regulator in circadian and developmental genes, immune-modulator, upto our knowledge. Timing application of luzindole (Melatonin receptor blocker) can cause the disruption in the growth of midgut and abdomen segments, such a way to control the development of nocturnal insect.

Publications

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2. S.P.Subala and **M.S.Shivakumar** (2017) Circadian variation affects the biology and digestive profiles of a nocturnal insect Spodoptera litura (Insecta: Lepidoptera) **Biological Rhythm Research**, 48(2): 207-226, DOI:10.1080/09291016.2016.1251928 **IF: 0.695**
3. S.P.Subala and **M.S.Shivakumar** (2018) Changes in light and dark periods affect the ArylalkylamineN-Acetyl transferase, melatonin activities and redox status in the head and hemolymph of nocturnal insect Spodoptera litura. **Biological Rhythm Research** 49:1, 13-28; https://doi.org/10.1080/09291016.2017.1325564 .2018 **IF: 0.695**
4. Karthi, S., Sankari, R., & **Shivakumar, M.S. 2014.** Ultraviolet-B light induced oxidative stress: Effects on antioxidant response of *Spodoptera litura* adults. **Journal of Photochemistry and Photobiology-B: Biology (Elsevier).** 135: 1–6. **IF: 3.035**
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