ORIGINAL ARTICLE

Physiological Implications of Prolonged Selenium Administrations in Normal and Desynchronized Adult Female Rats

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Abstract:

Background: Owing to the non-biodegradability of selenium, its prolonged consumption may lead to adverse health outcomes. *Aim and Objectives*: The study investigated the physiological effects of prolonged selenium administrations in normal and desynchronized rats. *Material and Methods*: Ninety six cyclical adult female rats were divided into short (1 week) and long (8 weeks) experimental cohort consisting of 6 groups each. Each experimental cohort contained control, High Selenium Dose (HSD) (150 µg/kg), Low Selenium Dose (LSD) (100 µg/kg), Desynchronized Group (AP), AP + HSD and AP + LSD. *Results:* In normal rats, HSD administration caused duration-dependent increase in ovarian PER1 expression and suprachiasmatic catalase and Glutathione Peroxidase (GPx) levels. LSD administration resulted in duration-dependent increase in Nocturnal Plasma Melatonin (NPM), ovarian PER1 expression, ovarian GPx and duration-dependent increase and decrease in nighttime temperature and ovarian catalase respectively. On the other hand, in AP rats, HSD administration resulted in duration-dependent increase in ovarian PER1, NPM and suprachiasmatic catalase and duration-dependent decrease in nocturnal plasma glucose and ovarian catalase respectively. Also, LSD administration led to duration-dependent decrease in ovarian GPX and increase in ovarian PER1, suprachiasmatic GPX and catalase levels respectively. *Conclusion:* In normal rats, 8-week administration of 150 µg/kg of selenium relatively improved ovarian PER1 expression and glutathione peroxidase and catalase levels in suprachiasmatic nucleus. Prolonged selenium administrations caused beneficial effects in desynchronized rats.

Keywords: Desynchronized, Selenium, Antiperoxidative Activity, Nocturnal Plasma Melatonin

Introduction:

With the discovery of electric bulb centuries ago, it is possible for human beings to be exposed to bright illumination courtesy of artificial light round the clock. In fact, occupational exposure to irregular lighting period is one of the major challenges associated with modern society [1, 2]. Indiscriminate or irregular exposure to artificial light results in desynchronization of physiologic functions [3, 4].

Studies are replete on the outcomes of light pollution-induced desynchronization in animals and humans. These include alteration in biorhythms causing perturbation in sleep/wakefulness cycle, daytime sleepiness, and narcolepsy [5], impaired body temperature rhythm [4], altered Luteinizing Hormone [LH] surge and

reproductive abnormality [6], deranged feeding cycle and obesity and disrupted melatonin rhythm [4, 7]. Exposure to light at night has been widely recognized as an important risk factor for breast cancer and metabolic disorders, specifically diabetes mellitus [8].

Apart from the circadian consequence of light pollution, Shang *et al.* (2014) [9] showed that exposure of Sprague Dawley rats to blue light resulted in apoptosis and necrosis of the photoreceptors. Exposure of Wistar rats to constant white light at 200 lux led to death of photoreceptor cells 5 days later [10]. A decrease in cellular viability and increases in cellular apoptosis and DNA damage following exposure to three light/dark cycles using Light Emitting Diode (LED) were also reported [10]. Light pollution also promotes retinitis pigmentosa and age-related macular degeneration [11-14] through photochemical, photothermal and photomechanical mechanisms. Other mechanisms of light pollution include promotion of oxidative stress in the retina and hypothalamus [10].

Suprachiasmatic nucleus is a part of the anterior hypothalamus that contains circadian pacemaker proteins, and these proteins play crucial roles in synchronizing other body internal clocks including mammary, uterine, hepatic, and ovarian clocks with the natural day/night cycle through neural and hormonal (melatonin) mechanisms [3]. Although, suprachiasmatic oxidative stress has been claimed to be involved in light pollution-induced desynchronization [10, 15], it is not clear whether persistent exposure to light pollution could cause a duration related changes in suprachiasmatic oxidative stress.

At least thirty percent of the world has been reported to be at risk of light pollution exposure consciously or unconsciously [16]. This therefore highlights the need for palliative–related scientific studies especially in the aspect of mitigating the adverse health consequences of erratic light exposure. In the past, precautionary measures regarding environmental conditioning, sleep hygiene education and melatonin regimen have been recommended [5]. Even though, with respects to chronobiological studies, fewer studies are available, the contribution of antioxidant minerals cannot be underestimated. Minerals such as zinc, copper and selenium have been implicated in the maintenance of health [17-18].

In the previous studies, selenium supplementations were reported to improve healing of gastric ulcer [19], lessen hypothyroid symptoms [20] and enhanced estrous cyclicity and ovarian anti-peroxidative activity in light deprived rats [21]. A study by Zhang and Zarbi, [22] indicated the chronobiological role of selenocysteine. However, in human and animal research, undesirable health outcomes have been reported to be associated with long term selenium consumption owing to its non-bioavailability [23]. Therefore, the need to understand the possible outcomes associated with long terms administration of selenium on not only rats desynchronized through exposure to light pollution became essential but also normal rats. The aim of the present study was to investigate the physiological effect of prolonged selenium administrations in normal and desynchronized rats.

Material and Methods:

Site of the Study:

The study was conducted in the department of Physiology, University of Benin, Benin-city Nigeria.

Animal Care and Management:

Ninety six cyclic female Wistar rats displaying 4-5 days estrous cycle length and weighing between 148-154 g were used for the research. They were purchased from the Department of Anatomy, University of Benin, Nigeria. They were housed in polypropylene (plastic) cages (37 cm ×27 cm) with stainless steel mesh cover. Rats were given grower mash and water through feeding and drinking troughs adlibitum. All rats were maintained in well ventilated chambers (156 cm×30 cm) as previously reported. Control chamber was under natural 12 hr light/12 hr dark cycle using digital lux meter. Rats in the control chamber were regularly maintained under natural 12 hr light/12 hr dark cycle.

Desynchronization of Rats:

Rats were desynchronized from natural daylight/ dark cycle by using artificial photoperiod-controlled chambers constructed using the method of Agoreyo and Adeniyi, (2018) [21]. Lighting in experimental chamber was provided by 8-watt fluorescent tubes at an illuminance of 120-150 lux. Lighting schedule in the experimental chambers was based on the method of Yorshinaka *et al.* (2016) [24]. Rats in the experimental chamber were maintained under alternate schedule of 20hr. light/ 4hr. dark.

Administrations:

Animal Treatments:

Rats were orally administered low and high doses of sodium selenite containing 100 µg/kg and 150 µg/kg according to the method of Jeong-Hwan *et al.* (2011) [25]. Administrations were done through oral gavage once per day for one week and eight weeks respectively.

Ethical Certification:

The study was approved by University of Benin Ethical Review Committee as a part of Postgraduate research (MED/294738).

Experimental Procedure:

The experiment was performed in accordance with National Institute of Health (NIH) guidelines for the care and use of laboratory animals. All rats were acclimatized under natural 12hr (light/dark cycle). They were randomly divided into short and long experimental cohorts, each consisting of 6 groups of 8 rats as shown in Table 1. The two experimental cohorts spanned for one and eight weeks respectively.

Table 1: Long and Short Experimental Cohorts

Groups	Administration	Light/Darkcycle	Light ON
Control	Distilled water (P.O.) (1 ml/300 g body weight) (P.O.) once per day	Natural 12 hr light/ 12 hr dark	7.00 am-7.00 pm
High selenium dose	1 ml/300 g body weight containing 150 µg/kg body weight of sodium selenite (P.O.) once per day	Natural 12 hr light/ 12 hr dark	7.00 am-7.00 pm

Continued...

Groups	Administration	Light/Darkcycle	Light ON
Low selenium dose	1 ml/300 g body weight containing 100 µg/kg body weight. of sodium selenite (P.O.) once per day	Natural 12 hr light/ 12 hr dark	7.00 am-7.00 pm
Altered lighting period	Distilled water (P.O) (1 ml/300 g body weight) once per day	20 hr light/ 4 hr dark in daily alternate schedule	7.00 am-3.00 am
Altered lighting period + high selenium dose	1 ml/300 g body weight containing 150 µg/kg body weight of sodium selenite (P.O.) once per day	20 hr light/ 4 hr dark in daily alternate schedule	7.00 am-3.00 am
Altered lighting period + low selenium dose	1 ml/300 g body weight containing 100 µg/kg body weight of sodium selenite (P.O.) once per day	20 hr light/ 4 hr dark in daily alternate schedule	7.00 am-3.00 am

Determination of Estrous Cycle Length:

Vaginal lavage was produced by gently infusing 0.1 ml of 0.9 percent saline solution 2-3 times into the vagina of the animals using a pipette. The pipette was then withdrawn, and the vaginal fluid collected was viewed using × 40 magnification lens of binocular microscope. Estrous cycle was classified into diestrus, proestrus, estrus and metestrus based on the cell type and density. Estrous cycle was calculated as the average number of days required for rats to migrate from proestrus to diestrus as previously reported [21, 26].

Nocturnal Blood Glucose:

The nocturnal blood glucose was determined using glucometer (Accu check, Roche Biochemical laboratory, Germany) in the night after taking the nighttime temperature.

Evaluation of Biorhythmic Synchronization:

Body Temperature Rhythm:

Daytime and nighttime body temperatures were monitored using mercury in glass thermometer from the vaginal by partially restraining the rats. The vaginal temperatures of the rats were obtained at 11.00 am (bathophase) and 8.00 pm (acrophase).

Plasma and Tissue Preparations:

The animals were euthanized after one and eight weeks respectively. In order to avoid estrous cycle-related fluctuations, only rats determined to be in estrus were euthanized and the process was carried out between 3.00 am to 5.00 am. Blood sample was collected through cardiac puncture into lithium heparin bottles. The blood samples were centrifuged to obtain the plasma and the plasma was separated into a plain bottle. Nocturnal melatonin was determined. The ovaries and suprachiasmatic structure were isolated. The right ovary was preserved in a bottle containing RNA later for ovarian PER1 expression study. The left ovary and suprachiasmatic structure were preserved in a solution of phosphate buffer for glutathione peroxidase and catalase assays.

Determination of Nocturnal Plasma Melatonin and Ovarian PER1 Expression:

Nocturnal Plasma Melatonin using Enzyme Linked Immunosorbent Assay (ELISA):

Fifty microliter standard was added to standard well and 40 µl of plasma to sample wells. Ten microliter anti-MT antibody was added to the sample wells and 50 µl streptavidin-HRP to both sample and standard wells. Incubation was done for 60 minutes at 37° C. Fifty microliter substrate solutions A and substrate solution B were added to each well respectively. Incubation was done for 10 min at 37° C in the dark. Stop solution was added to each well and the optical density value of each well was determined at 450 nm within 30 min of adding the stop solution.

Ovarian PER1 Expression using Real Time-Reverse Transcriptase Polymerase Chain Reaction:

After homogenization of ovarian tissue using mortar and pestle and lysate buffer, RNA extraction was performed using guanidinium thiocyanate-phenol-chloroform (trisol isolation kit). RNA purity and concentration were determined by NanoDrop 1000 Spectrophotometer. One microgram of ovary RNA was used for synthesis of cDNA using Taqman reverse transcription reagents in a total volume of 20 µl. The quantitative PCR was performed using mastermix (MX3000 Quantitative PCR System) with PER1 primers and Taqman probes. The forward and reverse primers used for PER1, according to Christiansen *et al.* [27] are:

F: AGCTCTGGAGACCACTGA

R: CACTCAGGAGACTATAGGCAATGGA

Fold expression was calculated relative to the house keeping gene (GAPDH) using delta-delta cycle threshold method ($2^{-\Delta\Delta ct}$).

Antiperoxidative Enzymes:

Glutathione Peroxidase (GPx) activity was measured by the procedure of Flohe and Gunzler, [28]. It was based on peroxidation of glutathione. Catalase activity was determined according to the method Sinha [29]. This method was based on the fact that dichromate in acetic acid is reduced to chromic acetate when heated in the presence of hydrogen peroxide with the formation of perchromic acid as an unstable intermediate.

Statistical Analysis:

All data were expressed as mean ± standard error of the mean (SEM) for 6 rats per group using SPSS 21. Statistically significant differences were accepted at $p<0.05$.

Results:

Effect of Desynchronization and Selenium Supplementations on Estrous Cycle Length, Nocturnal Blood Glucose and Body Temperature Rhythm:

Neither high nor low selenium dose administrations significantly caused duration-dependent effect on estrous cycle length in normal rats. There was a significant increase in estrous cycle length in desynchronized rats when compared with control group. In desynchronized rats, neither high nor low selenium dose administrations caused duration-dependent effect on estrous cycle length (Table 2). Neither high nor low selenium dose administrations significantly caused duration dependent effect in normal rats as far as nocturnal blood glucose was concerned. Desynchronized rats treated with high and low selenium doses for one week respectively exhibited significantly high nocturnal blood glucose when compared with control group. There was a duration-dependent decrease in nocturnal blood glucose in desynchronized rats treated with high selenium dose (Fig.1a). Daytime temperature

Table 2: Effect of Desynchronization and Selenium Administrations on Estrous Cycle Length

Groups	Short experimental cohort (Mean ± SEM) (days)	Long experimental cohort (Mean ± SEM) (days)
Control	4.2 ± 0.53	4.0 ± 1.37
High selenium dose	4.7 ± 0.88	4.6 ± 1.71
Low selenium dose	4.4 ± 1.77	4.8 ± 0.49
Desynchronized group	6.0 ± 0.45*	6.4 ± 1.64*
High selenium dose + desynchronization	5.5 ± 1.06	5.5 ± 1.04
Low selenium dose + desynchronization	5.0 ± 0.71	5.0 ± 0.62

significantly ($P<0.05$) decreased and increased after one week and eight weeks respectively in desynchronized rats when compared with control group. In rats administered distilled water, there was a duration-dependent decrease in daytime temperature. In desynchronized rats, neither high selenium dose nor low selenium dose administrations caused duration-related effect. However, in normal rats, low selenium dose administration caused a duration-dependent increase in nighttime temperature (Fig. 1b).

Effect of Desynchronization and Selenium Supplementations on Nocturnal Plasma Melatonin and Ovarian PER1 Expression:

In normal rats, low selenium dose administration caused a duration-dependent increase in nocturnal plasma melatonin. Desynchronized rats showed a duration-dependent decrease in nocturnal plasma melatonin level. In desynchronized rats, administration of high selenium dose led to a duration dependent increase in plasma melatonin (Fig. 2a).

At high and low selenium doses administrations, there was a duration dependent increase in ovarian PER1 expression in normal rats. There was a significant decrease in ovarian PERI expression in desynchronized rats for one week and eight weeks respectively when compared with control group. There was duration dependent increase in ovarian PERI expression in desynchronized rats treated with high and low selenium doses respectively (Fig. 2b).

Effect of Desynchronization and Selenium Administrations on Ovarian Antiperoxidative Enzymes:

In normal rats, low selenium dose administration caused a duration dependent increase in ovarian glutathione peroxidase. In desynchronized rats, selenium administration at high dose significantly increased the ovarian glutathione peroxidase after one week and eight weeks respectively when compared with control group. There was duration-dependent decrease in ovarian glutathione peroxidase in desynchronized rats treated with low dose of selenium (Fig. 3a). In normal rats, low selenium dose administration led to duration dependent decrease in ovarian catalase. There was a duration-dependent decrease in ovarian catalase in desynchronized rats treated with high selenium dose. There was no duration dependent effect in ovarian catalase in desynchronized rats treated with high selenium dose (Fig. 3b).

Effect of Desynchronization and Selenium Administrations on Suprachiasmatic Anti-peroxidative Enzymes:

In normal rats, administration of high selenium dose led to duration-dependent increase in suprachiasmatic glutathione peroxidase. There was a duration dependent increase in suprachiasmatic glutathione peroxidase in desynchronized rats treated with low selenium dose (Fig. 4a).In normal rats, high selenium dose administration led to duration dependent increase in suprachiasmatic catalase. There was a significant decrease ($P<0.05$) in suprachiasmatic catalase in desynchronized rats eight weeks when compared with control group. There was a duration-dependent increase in suprachiasmatic catalase in desynchronized rats treated with high and low selenium doses (Fig. 4b).

Relationship between Ovarian PER1 Expression and Estrous Cycle Length:

There was a strong negative correlation between ovarian PER1 expression and estrous cycle length (Table 3).

Relationship between Ovarian PER1 and Antioxidant Parameters:

There was a strong positive correlation between ovarian PER1 and hypothalamic glutathione peroxidase. There was also a positive correlation between ovarian PER1 and hypothalamic catalase. There was also a positive correlation between ovarian PER1 and melatonin (Table 4).

Relationship between Melatonin and Anti-peroxidative Enzymes:

There was a strong positive correlation ($P<0.05$) between melatonin and ovarian glutathione peroxidase. There was a positive correlation ($P<0.05$) between melatonin and hypothalamic glutathione peroxidase. There was a positive correlation ($P<0.05$) between melatonin and hypothalamic catalase (Table 5).

Table 3: Correlational Analyses between Ovarian PER1 and Estrous Cycle Length

r	Estrous cycle length
Ovarian PER1	-0.586*

**P<0.05*

Table 4: Correlational Analyses between Ovarian PER1 and Antioxidant Parameters

r	Ovarian GPx	Ovarian catalase	Suprachiasmatic GPX	Suprachiasmatic catalase	Melatonin
Ovarian PER1	0.140	0.128	0.756*	0.454*	0.375*

**represents P<0.05*

Table 5: Correlational Analyses between Melatonin and Antiperoxidative Enzymes

r	Ovarian GPx	Ovarian catalase	Suprachiasmatic GPX	Suprachiasmatic catalase
Melatonin	0.705*	0.314	0.423*	0.415*

**represents P<0.05*

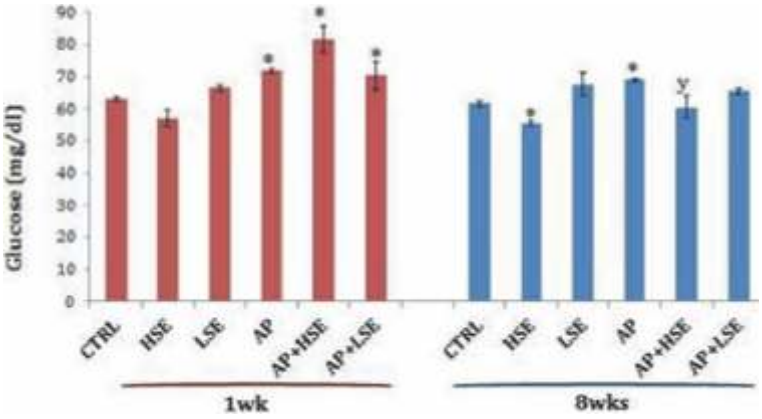

Fig. 1a: Effect of Desynchronization and Selenium Administrations on Nocturnal Blood Glucose. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from Ctrl at P<0.05. [y]Represents Significant Difference (P<0.05) from 1 wk.

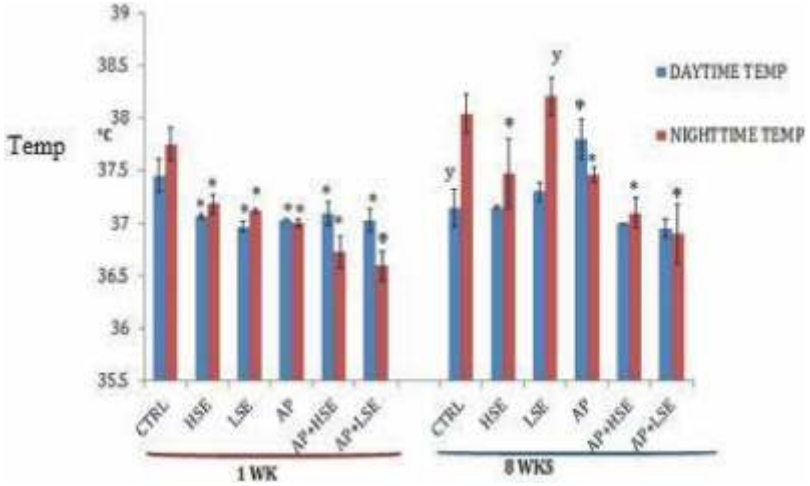

Fig. 1b: Effect of Desynchronization and Selenium Administrations on Body Temperature Rhythm. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from Ctrl at P<0.05. [y]Represents Significant Difference (P<0.05) from 1 wk. [a]Represents Significant Difference (P<0.05) from AP.

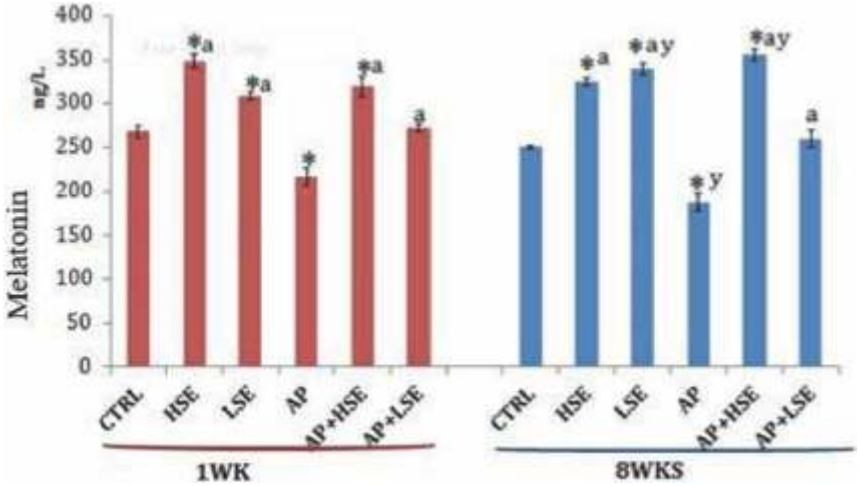

Fig. 2a: Effect of Desynchronization and Selenium Administrations on Nocturnal Plasma melatonin. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from Ctrl at P<0.05. [y]Represents Significant Difference (P<0.05) from 1 wk. [a]Represents Significant Difference (P<0.05) from AP.

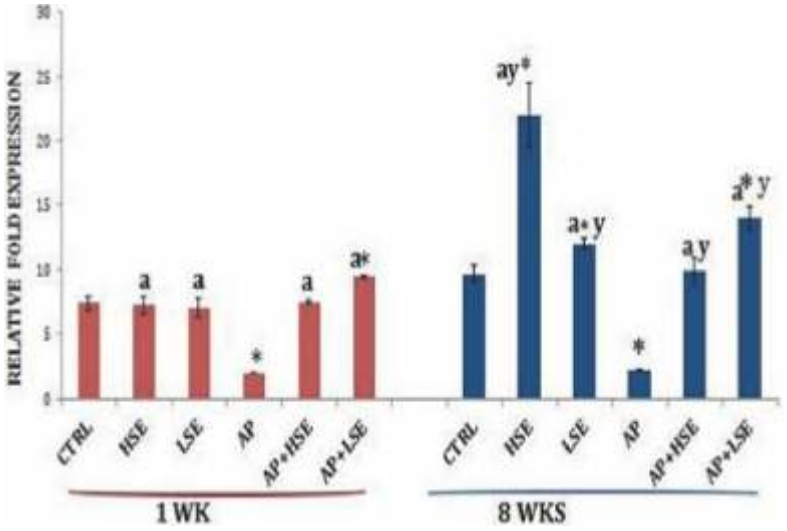

Fig. 2b: Effect of Desynchronization and Selenium Administrations on Ovarian Circadian PER1. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from ctrl at P<0.05. [y]Represents Significant Difference (P<0.05) from 1 wk. [a]Represents Significant Difference (P<0.05) from AP.

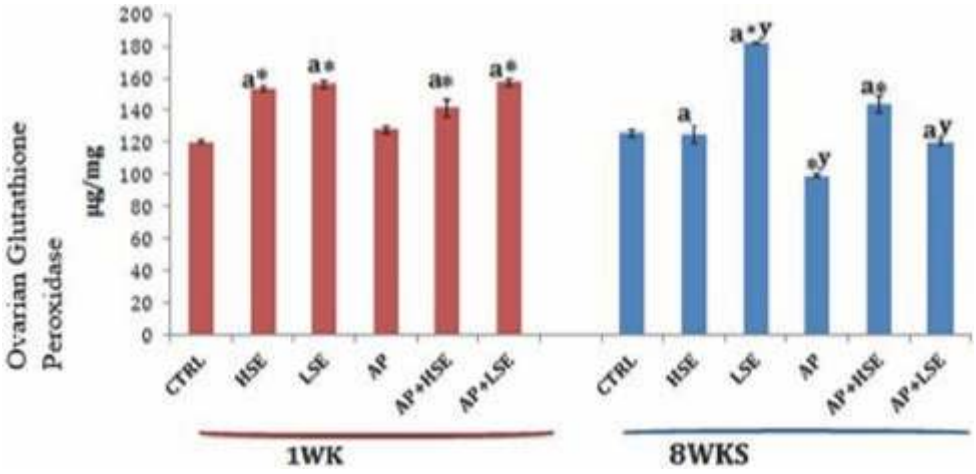

Fig. 3a: Effect of Desynchronization and Selenium Administrations on Ovarian Glutathione Peroxidase. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from Ctrl at P<0.05. [y]Represents Significant Difference (P<0.05) from 1 wk. [a]Represents significant difference (P<0.05) from AP.

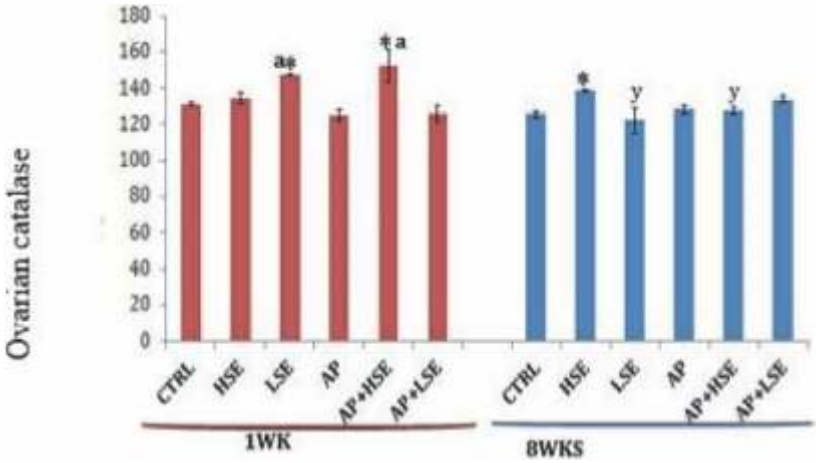

Fig. 3b: Effect of Desynchronization and Selenium Administrations on Ovarian Catalase. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from Ctrl at P<0.05. [y]Represents Significant Difference (P<0.05) from 1 wk. [a]Represents Significant Difference (P<0.05) from AP.

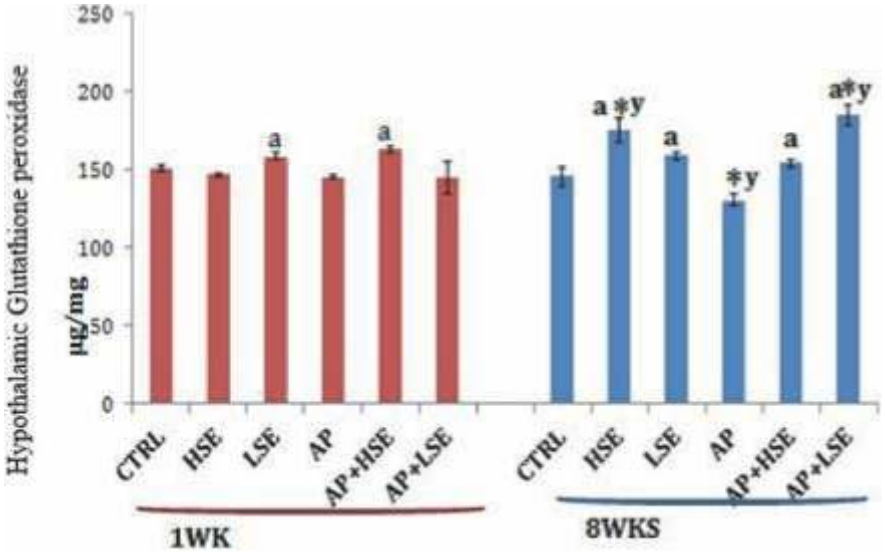

Fig. 4a: Effect of Desynchronization and Selenium Administrations on Supachiasmatic Glutathione Peroxidase. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from Ctrl at P<0.05. [y]Represents Significant Difference (P<0.05) From 1 Wk. [a]Represents Significant Difference (P<0.05) From AP.

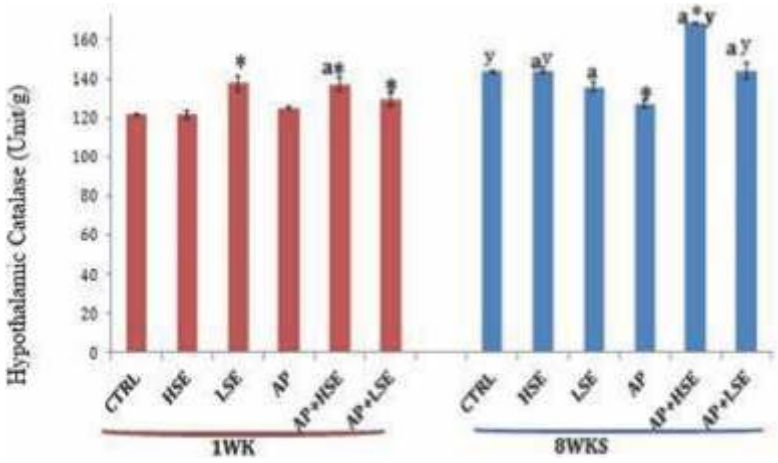

Fig. 4b: Effect of Desynchronization and Selenium Administrations on Suprachiasmatic Catalase. CTRL-Control, HSE-High Selenium Dose, LSE-Low Selenium Dose, AP-Desynchronized Group. [*]Represents Significant Difference from Ctrl atP<0.05. [y]Represents Significant Difference (P<0.05) from 1 Wk. [a]Represents Significant Difference (P<0.05) from AP.

Discussion:

Irregular lighting exposure is widely known to alter biorhythms, disrupt circadian rhythms and orchestrate desynchronization [4-5]. The melatonin-boosting effect of selenium supplementation has been previously reported [29] and the adverse effect of selenium following long administration has been documented [23]. The present study examined the physiological implications of selenium administration in normal and desynchronized rats.

Estrous cycle length gives information about the time taken for the completion of rats' reproductive cycle [21]. In the study, selenium administrations exerted no duration-related effect on estrous cycle length in both normal and desynchronized rats even though irregular lighting induced desynchronization prolonged estrous cycle length. Unlike estrous cycle length, high selenium dose administration led to a duration-dependent decline in nocturnal blood glucose. The finding highlights the potential palliative role of selenium especially in women who are at risk of erratic light exposure such as night shift workers. Light at night has been penned as a risk factor for glucose imbalance [7].

Selenium administrations at both doses on daytime and nighttime body temperature in desynchronized rats did not result in duration-related effect. This result was very significant as it indicated that the effect of selenium doses on body temperature in desynchronized rats was independent of frequency of treatment. Secondly, it highlighted the level of sustainability associated with selenium use as a chronobiotic agent. Conversely, in normal rats, only low selenium dose administration resulted in increased nighttime temperature. In desynchronized female rats, there was impairment in the rhythm of body temperature. The result agreed with data from night workers [5, 30]. Although, rats are known to be nocturnal, the increase in daytime body temperature observed in desynchronized rats for eight weeks and the duration-dependent decrease in daytime temperature seen in animals administered distilled water might indicate adaptation of peripheral internal clocks to other external cues such as ambient temperature and anthropogenic factors [31]. Mechanisms underlying disruption in body temperature rhythm might include repression of circadian gene [5, 30] such as PERiod (PER), Circadian Locomotor Oscillation Cycles of Caput (CLOCK) and Brain and Muscle Arnt Like Protein (BMAL-1) [7, 32-33].

Increase in ovarian antiperoxidative enzymes (glutathione peroxidase and catalase) following selenium administrations was necessary probably to protect the released secondary oocyte and remnants of graafian follicle from oxidative damage by free radicals [34-35]. In our study, all rats were euthanized at estrus phase and studies have shown that during estrous cycle, antioxidant activity in ovary homogenate was low in the estrus and proestrus phases [35-36]. In the study, low selenium doses administration caused duration-dependent increase and decrease in ovarian levels of glutathione peroxidase and catalase respectively. On the other hand, high selenium doses administration caused duration dependent decrease in ovarian catalase and glutathione peroxidase in desynchronized rats respectively. This depression might be due autoregulatory tissue response.

High and low selenium dose increased suprachiasmatic glutathione peroxidase and catalase. We observed that selenium administration at 150 µg/kg caused a duration-dependent increase in suprachiasmatic glutathione peroxidase and catalase but had no duration-related effect on suprachiasmatic glutathione peroxidase in desynchronized rats. However, in desynchronized rats there was duration-dependent increase in hypothalamic catalase. The increase in suprachiasmatic antiperoxidative enzymes orchestrated by selenium administration implies the potentials of the chemical in mitigating oxidative stress. Oxidative stress is one of the underlying mechanisms of circadian desynchronization and neural damage [10, 15].

Like other studies [1, 8], desynchronization through irregular lighting period exposure has been shown to decrease nocturnal melatonin synthesis. However, in our study a peak secretion occurred after one week of altered lighting-induced desynchronization. The tendency of light to suppress nocturnal melatonin synthesis is known as the negative masking effect of light [37]. Suppressed nocturnal melatonin secretion typified impaired synchronization of physiological rhythms with external rhythm [30].

Although, we are aware that supplementations with tryptophan [38] and pyridoxine [39] increased nocturnal melatonin secretion, there is insufficiency of information both in human and animal studies expressing the possibility of selenium supplementations improving nocturnal melatonin secretion. In the present study, we showed that selenium administration at high dose and low dose enhanced melatonin secretion in desynchronized rats and normal rats respectively. In desynchronized rats, nocturnal plasma melatonin secretion increased with frequency of high selenium dose administration. The result highlighted the time-dependent promising role of selenium with respect to remediation of conditions associated with circadian misalignments.

Glutathione peroxidase is a selenoprotein and its level can be used as an index of selenium concentration in the blood [16]. The results of the study indicated that melatonin positively correlated with suprachiasmatic levels of glutathione peroxidase and catalase. However, the strong positive correlation between the ovarian glutathione peroxidase and melatonin indicated that selenium administration may relatively boost melatonin secretion. Previous studies, most especially by Venkateswaran *et al.* (1996) [40] has reported antioxidant synergism between selenium and vitamin E in the prevention of *in-vitro* prostate cancer cell growth.

Our study also exhibited sufficient evidence to support the chronobiotic property of melatonin. For example, the positive correlation between melatonin and ovarian PER1 demonstrated the synchronization of ovarian circadian clock with external cue such as natural light/dark cycle as previously reported by many investigators including Hardeland *et al.* (2004) [41] and McMillian *et al.* (2006) [42].

Irregular lighting-induced desynchronization was associated with depressed ovarian PER1 expression. Studies most especially by Coomas *et al.* (2012) [37] indicated that irregular lighting pattern dampened PER 2 expressions in the suprachiasmatic nucleus. Besides the fact that we

chose PER1 due to its light sensitivity, we have simply demonstrated perturbation of peripheral circadian clock by altered lighting period. Depression of ovarian PER1 expression observed in rats desynchronized through exposure to irregular lighting period might be due to down-regulatory influence of stimulus overload on gene transcription [43]. We also observed that the suppressive effect of altered lighting period on ovarian PER1 was not duration dependent.

Early study by Zhang and Zarbi (2009) [44] showed that selenium supplementation prevented nitroso *N*-methyl urea induced mammary carcinogenesis in rats through increased expression of PER2. It was observed that in normal and desynchronized rats, ovarian PER1 expression increased as the frequency of selenium administrations increased. This finding was very significant as it provided an additional insight into the possibility of using selenium as a time-bound nutritional regimen in the management of oncological diseases.

The strong negative correlation between ovarian PER1 expression and estrous cycle length showed that the lower the ovarian PER1 expression, the longer the estrous cycle. Longer estrous cycle occurs in anovulation, and it is characterized by increase in estrous cycle ratio [4, 34]. Furthermore, the positive correlation between ovarian PER1 and hypothalamic antiperoxidative enzymes indicate not only existence of a nexus between hypothalamic antioxidant homeostasis and circadian expression in peripheral tissue, the ovary. Although the physiological importance of this connection is not well understood, predicating on the finding of He *et al.* (2007) [45], it is possible that the FSH induced increase in ovarian PER1 expression was mediated by increase in hypothalamic antiperoxidative activity. Selenium-induced increase in tissue antiperoxidative activity observed in our study agreed with previous reports [16, 46]. The report of Jockers *et al.* (2016) [47] claimed there exists antioxidant synergism between melatonin and antioxidant enzyme. In addition to our previous study [48], further investigations will be needed to clarify whether there is an existence of synergism between melatonin and suprachiasmatic antiperoxidative enzymes and the possible implication of this on ovarian clock.

For non-biodegradable chemicals like selenium, studies meant to understand effect of frequency of exposure is inevitable. The present study provided information about the impact of treatment frequency on chronobiological and antiperoxidative effect of selenium in desynchronized rats. The findings of the study remain significant as long as attention towards the might of non-drug therapy in the management of adverse consequences of circadian misalignment continues to increase.

Conclusion:

The results of the study showed that 8-week administration of 150 µg/kg of selenium relatively improved ovarian PER1 expression and suprachiasmatic glutathione peroxidase and catalase in normal rats. Prolonged selenium administrations caused beneficial effect in desynchronized rats.

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