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Short day photoperiod protects against acetaminophen-induced heptotoxicity in rats

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Abstract
This study investigated the effect of different photoperiods on acetaminophen-induced hepatotoxicity in rats. Twenty four adult male rats (average weight = 160±7g) were conditioned to different photoperiod regimens for 6 weeks. At the end of the 6-week period, rats exposed to normal, short and long photoperiods received oral acetaminophen (2g/kg body weight) while in the control group, exposed to normal photoperiod, received oral saline. Rats were sacrificed 24 h after acetaminophen administration by cervical dislocation and blood was collected by cardiac puncture for the estimation of liver enzymes activities. Liver tissues were excised and homogenized for estimation of liver malondialdehyde (MDA) concentration. Elevation of serum levels of alanine and aspartate transaminases and alkaline phosphatase caused by acetaminophen intoxication were not affected in rats subjected to long photoperiod while these parameters were significantly (P<0.05) reduced in rats subjected to short photoperiod. However, alteration of photoperiod resulted in significantly (P<0.05) lower serum gamma glutamate transpeptidase and total protein in acetaminophen-treated rats. All groups of rats had similar serum albumin while serum malondialdehyde concentration was significantly lower in rats subjected to short photoperiod. This study revealed the protective effects of short photoperiod against acetaminophen-induced hepatotoxicity and lipid peroxidation in rats.

Introduction

Studies have shown that short day lengths generally enhance many types of immune responses in the laboratory animals (Nelson, 2004; Olayaki *et al.*, 2008). Increased cell-mediated immune responses including lymphocyte proliferation to mitogens, delayed-type hypersensitivity responses and overall numbers of circulating immune cells have been reported as a consequence of exposure to short day length (Blom *et al.*, 1994; Demas and Nelson, 1996; Demas and Nelson, 1998; Bilbo *et al.*, 2002). Photoperiodic adjustment in immune defences is mediated almost entirely by the pineal melatonin rhythm. Melatonin, a hormone produced by a wide range of organism including animals, plants and microbes, has been reported for its anti-inflammatory and immune enhancing actions in laboratory animals (Maestroni *et al.*, 1987; Guerrero and Reiter, 2002). Its effects as a free radical scavenging agent have also been reported (Tan *et al.*, 2002; Reiter *et al.*, 2002; Rodriquez *et al.*, 2004). Previous studies have shown that melatonin exhibited hepatoprotective effect by reducing the generation of reactive oxygen species and lipid peroxidation (Kanno et al., 2006). Mathes (2010) showed that hepatoprotective effect of melatonin was mediated by melatonin receptors that present in the hepatocytes. Although melatonin is synthesized in a number of tissues such as retina, GIT, lymphocytes, circulating melatonin in mammals is largely derived from the pineal gland (Cardinali and Pevet, 1998). Pineal melatonin production exhibits a circadian rhythm, with a low level during daytime and high levels during night (Claustrat *et al.*, 2005).

This study, investigated the effect of changes in photoperiod exposure on liver enzymes and lipid peroxidation following acetaminophen hepatotoxicity in rats. Paracetamol (acetaminophen), widely used

antipyretic and analgesic, is mainly metabolised in the liver to excretable glucoronide and sulphate conjugates (Jallow *et al.*, 1974; Wong *et al.*, 1981). Despite this therapeutic usage, hepatotoxic actions of acetaminophen are well established (James *et al.*, 2003). Acetaminophen is converted in the presence of cytochrome P-450 to its reactive metabolite, N-acetyl-p-benzoquinone imine (NAPQI), which covalently binds to cysteine to form a conjugate with glutathione to deplete glutathione stores. This results in the accumulation of NAPQI and mitochondrial dysfunction leading to the development of acute hepatic necrosis in liver centrolobular cells (Jaeschke *et al.*, 2002). Though the hepatoprotective effect of melatonin is well-established, the relationship between photoperiods and the protective actions of melatonin is not well understood. Therefore, this study was carried out to determine how different photoperiods affect the hepatoprotective action of melatonin in acetaminophen-induced liver damage in rats.

Materials and Methods

Experimental animals: Twenty four male Sprague Dawley rats were obtained from the Animal Breeding Unit of the Department of Biochemistry, University of Ilorin, Ilorin, Nigeria. The rats weighed 160±7g and were conditioned to different light conditions for 6 weeks. All animals were housed in plastic cages with stainless steel mesh cover under standard laboratory conditions in photoperiod-controlled chambers. Lighting in photoperiod chambers was provided by 6-watt fluorescent tubes at illuminance of 100-250 lux located at 5cm above each cage. The experiment was conducted during the raining season. Rats pellet (Bendel Feeds, Edo State, Nigeria) and water were provided *ad libitum*. All experimental procedures were approved by the Animal Ethical Committee of the Faculty of Basic Medical Sciences, University of Ilorin.

Experimental design: Animals were divided into 4 groups (n=6) and subjected to photoperiodic conditions of light/dark cycle as shown in Table 1 for 6 weeks. At the end of the treatment period, rats in groups II, III and IV received oral administration of 2g/kg body weight of acetaminophen (Mayer and Baker Nigeria PLC, Ota, Ogun State, Nigeria) suspension in normal saline as previously described (Rajesekaran and Periyasamy, 2012; Rekha et al., 2013). The rats were sacrificed 24 hr after acetaminophen administration by cervical dislocation. Blood samples were collected by cardiac puncture and serum separated by centrifugation. Liver of sacrificed animals were excised immediately and homogenized for the determination of tissue concentration for malondialdehyde.

Table 1: Photoperiod exposure of normal and acetaminophen-treated rats

Groups	Saline Control	Acetaminophen Control	Long Photoperiod	Short Photoperiod
Light/Dark Cycle (hrs)	12:12h	12:12h	16:8h	8:16h
Time of the day	07:00-19:00h	07:00-19:00h	05:00-21:00h	09:00-17:00h

Rats in acetaminophen control group as well as long and short photoperiod groups received a single oral administration of 2g/kg body weight of acetaminophen following 6 weeks exposure to different photoperiods

Assessment of liver enzyme activities: Serum activities of alanine aminotransferase (ALT), alkaline phosphate (ALP), aspartate aminotransfrase (AST) and gamma glutamate transpeptidase (GGT) as well as concentrations of total protein and albumin levels were measured using the commercially available assay kits (Agape Diagnostics, Switzerland Gmbh) following the manufacturer's recommended procedures.. Assays performed using an automated blood chemistry analyser (URIT-810 Chemistry Analyzer, URIT Medical Electronic Co., Ltd. Guangxi, PR China).

Assessment of lipid peroxidation: Tissue malonaldedyde (MDA) levels were estimated as described by Ohkawa *et al*, (1979). Tissues were homogenized in ice cold KCl (100mM) containing 0.003M EDTA (3ml) and centrifuged at 600g for 15min. Aliquots of supernatant (400µl) were added to a solution containing 8.1% SDS, 20% acetic acid (pH 3.5), 0.8% thiobarbituric acid and 0.6ml water and heated at 95°C for 60 minutes. This was followed by the addition of 1.0ml water and 5.0ml of n-butanol-pyridine mixture (15:1, vol/vol). The mixture was vigorously shaken and centrifuged at 2,000g for 15minutes. The absorbance of the upper layer was read at 532nm using an AcurexMicroplate Reader. Malondialdehydebis-dimethyl acetal was used as the external standard and results were expressed as nanomoles MDA per milligram protein. The intra-assay variability was determined in five sets of triplicate samples and the coefficient of variation given.

Statistical Analysis: Data were analysed using GraphPad Prism Version 3 and expressed as mean ± SEM. More than two variables were compared by using one-way ANOVA test and

Duncan's test was performed for post-hoc analysis.

Results

Effects of photoperiods on serum levels of liver function parameters in acetaminophen-treated rats: Administration of acetaminophen resulted in marked elevation of serum levels of AST (496%, $P<0.001$), ALT (389%, $P<0.001$), ALP (99%, $P<0.001$) and GGT(255%, $P<0.001$) compared with control animals treated with saline (Figure 1). Exposure to long photoperiod (16:8, light:dark hr) produced no significant effect on serum levels of AST , ALT and ALP but inhibited increased serum levels of GGT caused by acetaminophen administration by 26% ($P<0.05$). However, significant reduction in serum levels of AST (40%, $P<0.01$), ALT (28%, $P<0.05$) and GGT (74%, $P<0.001$) and no change in serum ALP were observed in rats exposed to short photoperiod. Serum total protein increased by 5-fold ($P<0.001$) in acetaminophen-treated rats compared with their saline-treated counterparts (Figure 1E). This increase was reduced by 37% ($P<0.05$) and 46% ($P<0.05$) in rats exposed to long and short photoperiods respectively. However, similar serum levels of albumin was observed in all groups of rats (Figure 1F).
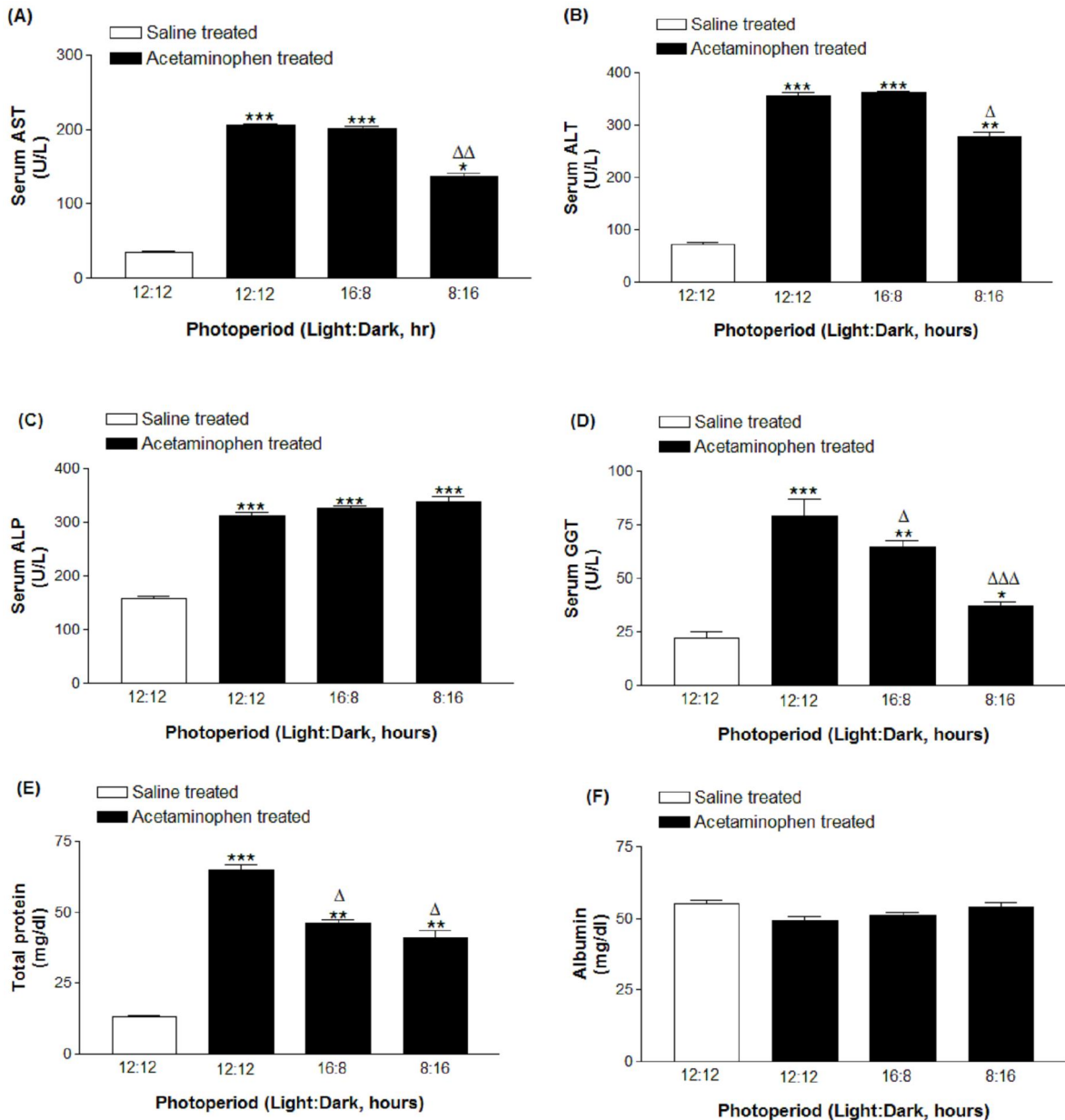
Figure 1.

Figure 1: Effects of different photoperiods on serum levels of (A) aspartate transaminase, (B) alanine transaminase, (C) alkaline phosphatase, (D) gamma glutamate transpeptidase, (E) total protein and (F) albumin in rats with acetaminophen-induced hepatotoxicity. Values are mean ± SEM with n=6. *$P<0.05$, **$P<0.01$ and ***$P<001$ compared with saline treated rats exposed to 12:12 light:dark photoperiod. $\Delta P<0.05$, $\Delta\Delta P<0.01$, $\Delta\Delta\Delta P<0.001$ compared with acetaminophen treated rats exposed to 12:12 light:dark photoperiod.

Effects of photoperiods on lipid peroxidation in acetaminophen-treated rats: Basal serum malondialdehyde concentration in saline-treated rats exposed to 12 h light and 12 h dark cycle was 172.5 mmol/mg proteins. This value was increased by 2.1-fold (P<0.001) in acetaminophen-treated rats under similar experimental conditions (Figure 2). Exposure to long photoperiod had not significant effect on serum concentration of malondialdehyde but rats maintained under short photoperiod showed a 35% (P<0.05) reduction in serum malondialdehyde level.

Figure 2.

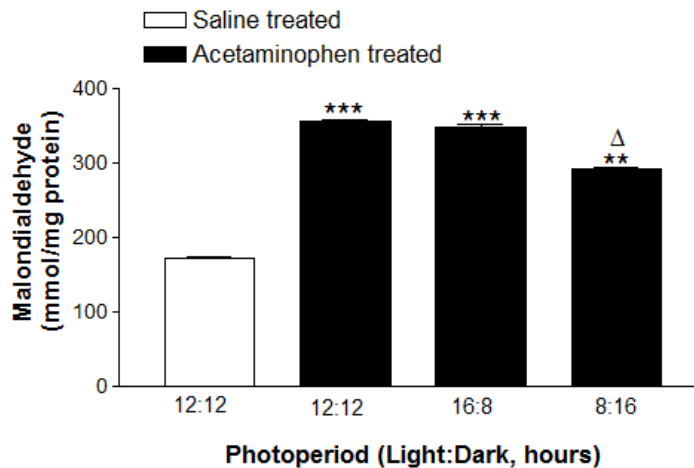Figure 2: Effects of different photoperiods on serum levels of malondialdehyde in rats with acetaminophen-induced hepatotoxicity. Values are mean ± SEM with n=6. **P<0.01 and ***P<001 compared with saline treated rats exposed to 12:12 light:dark photoperiod. $^{\Delta}$P<0.05, compared with acetaminophen treated rats exposed to 12:12 light:dark photoperiod.

Discussion

Acetaminophen containing drugs are commonly used globally for the treatment of pain and fever (Kaufman *et al*, 2002). Despite this important therapeutic utility, studies have shown that its consumption at doses exceeding the maximum recommended dose of 4 g/day could result to serious liver injury or death in humans (Watkins and Seef, 2006). Studies in laboratory animals have also confirmed that administration of acetaminophen at doses in excess of 2g/kg body weight could result in significant liver injury (Madkour and Abdel-Daim, 2013). These reports are consistent with public health concerns raised by the increasing incidence of acetaminophen-related liver injury over the past few years (Kaufman *et al*, 2002). Acetaminophen is converted to its toxic metabolite, N-acetyl-P benzoquinone imine. This oxidation product of acetaminophen binds to sulphydryl groups of protein in the liver to induce cell necrosis and lipid peroxidation caused by decreased hepatic gluthathione levels (Boyd *et al.*, 1966). Increase in circulating concentrations of liver enzymes (ALT and AST), which are of cytoplasmic origin, is indicative of hepatic injury (Drotman *et al*, 1978). Increased serum ALP and γ-glutamyl transpeptidase levels, resulting from increased ALP synthesis in the presence of increasing biliary pressure, has been recognized as a more specific and sensitive biomarkers of liver injury (Muriel and Garcipiana, 1992).

In this study, rats intoxicated with acetaminophen exhibited characteristics features that are consistent with previous reports of its ability to induce liver damage (Galal *et al*, 2012; Madkour and Abdel-Daim, 2013). Serum levels of AST, ALT, ALP and GGT were significantly elevated in rats intoxicated with acetaminophen under normal 12:12 light:dark photoperiods. This impaired liver function is also accompanied with elevated serum levels of malondialdehyde, which suggests increased lipid peroxidation. Increased lipid peroxidation in acetaminophen toxicity is believed to be a consequence of increased interaction between cellular lipids and acetaminophen metabolites (Albano *et al*, 1983). This observation is consistent with previous studies reporting decreased cellular reduced glutathione activities, increased malondialdehyde production and promotion of lipid productions in both in vitro and in vivo models of acetaminophen-hepatotoxicity (Fairhurst *et al*, 1982; Albano *et al*, 1983).

The result of this study has clearly shown that altering photoperiod effectively protects

against the deleterious effects of acetaminophen on the liver. Our results indicated that increasing light period from 12 to 16 hours daily produced no significant effect on the serum concentrations of AST, ALT or ALP but produced a significant reduction in the circulating concentrations of GGT and reduced the production of malondialdehyde. However, a more pronounced inhibition of the deleterious effects of acetaminophen, exemplified by significant reduction in serum concentrations of liver enzyme and malondialdehyde was observed in rats treated with 8 hours of light and 12 hours of dark period for 6 weeks prior to acetaminophen intoxication. Results obtained in rats subjected to 8:12 light:dark period are in agreement with previous studies indicating a reduction in serum levels of liver enzyme and malondialdehyde with reduced acetaminophen-induced hepatotoxicity and lipid peroxidation in vivo (Madkour and Abdel-Daim, 2013).

Though the exact mechanism by which changes in photoperiod affect acetaminophen-induced hepatotoxicity is not yet fully understood, several studies have reported the effect of changes in photoperiod on signal transduction in animals (reviewed in Dahl *et al*, 1999). Studies have shown that photoperception occurs in the retina in mammals, leading to the stimulation of retinal photoreceptors which send inhibitory signals to the pineal gland and modulate its secretion of several hormones, including melatonin (Reiter, 1991). Studies have shown that light inhibits the activity of N-acetyltransferase, the rate-limiting enzyme in melatonin synthesis (Illnerova and Sumova, 1997). This observation suggests that the reduction of light phase from 12 hto 8 h in this study may be accompanied by increased melatonin secretion. In addition to its ability to regulate the sleep/wake cycle in mammals, antioxidant and anti-inflammatory effects of melatonin have also been reported (Esposito and Cuzzocrea, 2010; Morcelli *et al*, 2013). Li *et al* (2014) also reported that melatonin protects against hepatic ischemia/reperfusion injury in clinical liver disease.

Melatonin binds to melatonin receptor 1 or 2 to elicit downstream signalling cascades with various physiologic effects. Melatonin receptors are G protein-coupled receptors which are expressed in various tissues of the body, including several parts of the central nervous system (CNS), ovary, retina, testis, mammary gland, coronary circulation and aorta, gall bladder, kidney, skin, adpocytes and the liver (Sallinen *et al*, 2005, Mathes *et al*, 2008). The activation of melatonin receptor type 1A by melatonin or melatonin receptor agonists leads to the inhibition of the cAMP signalling pathway via the inactivation of adenylate cylcase followed by reduced protein kinase A (PKA) activity and decreased generation

of phosphorylated cAMP responsive element-binding protein (P-CREB) (Boutin *et al*, 2005; Ma *et al*, 2008). In addition, the activation of melatonin receptor 1 potentiates the release of arachidonate following the activation of phospholipase C and regulate ion flux inside the cell (Ma *et al*, 2008). The activation of melatonin receptor type 2 inhibits adenylyl cyclase s well as guanylyl cyclase and therefore affects the production of both cAMP and cyclic guanosine monophosphate (cGMP) (Hattori *et al*, 1995; Ma *et al*, 2008). Though the molecular mechanisms through which melatonin exerts its protective effects against acetaminophen intoxication as observed in this study is not yet clear, we hypothesize that the expression of melatonin receptors in rat liver may play a significant role. We also hypothesize that during the short photoperiod, melatonin secretion is increased in rats used in this study over the six weeks period. This elevated melatonin concentration resulted in enhanced protection of these animals against acetaminophen-induced lipid peroxidation and liver damage.

Consistent with previous studies (Yakubu et al, 2013), oral administration of acetaminophen has no significant effect on serum albumin level but resulted in significantly elevated serum total protein concentration. This result negates the usual observation of reduced serum protein in liver damage (Oyagbemi and Odetola et al, 2010; Ekam et al, 2012) and strongly suggests that the administration of acetaminophen in this study may affect metabolic processes such as bone marrow development and the development of other conditions such as multiple myeloma, which contribute to elevated serum protein (O'Connell et al, 2005; Walter et al, 2011). Changes in photoperiod examined in this study did not affect serum albumin levels. However, reduced total serum protein were observed in rats exposed to long photoperiod while increased total serum protein was observed in rats exposed to short photoperiod. Increased serum total protein has been reported in hepatic damage (Melligen and Wergeland, 2000). However, it is not yet clearly understood how changes in photoperiod affect serum protein in acetaminophen-induced hepatotoxicity.

Conclusion

In conclusion, this study have shown that exposure to short photoperiod for 6 weeks offer some protection against acetaminophen-induced lipid peroxidation and liver damage in rats via a mechanism that is believed to involve enhanced production of melatonin. This observation encourages further investigations of melatonin-mediated roles in the protection against liver injury by short photoperiod as a way of identifying its therapeutic utility.

Conflict of Interests
Authors declare no conflict of interests.

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