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Temporal Patterns of Blood Lipidperoxides and Antioxidants in 7,12-dimethylbenz(a)anthracene Induced Hamster Buccal Pouch Carcinogenesis

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Abstract: Our aim was to investigate the temporal patterns of lipid peroxidation byproducts [thiobarbituric acid reactive substances (TBARS)] and enzymatic antioxidants [superoxide dismutase (SOD), catalase (CAT) and glutathione peroxidase (GPx)] in 7,12-dimethylbenz(a)anthracene (DMBA) induced hamster buccal pouch carcinogensis. The levels of thiobarbituric acid reactive substances and the activities of antioxidant enzymes were assayed at 4 h intervals throughout the 24 h period using colorimetric methods. The Cosinorwin computer software program was used to analyse the characteristics of biochemical rhythms such as acrophase, amplitude and mesor etc., Delayed acrophase was noticed for lipid peroxides and antioxidant enzymes in hamsters with oral squamous cell carcinoma as compared to control animals. Elevated and declined mesor values for TBARS and enzymatic antioxidants, respectively were observed in cancer animals. The disrupted diurnal rhythms of TBARS and antioxidants observed in the present study reflected an alteration of circadian clock function for these biochemical variables in DMBA induced oral carcinogenesis.

Key words: Diurnal rhythm, oral carcinogenesis, lipidperoxidation, antioxidants

INTRODUCTION

Oral squamous cell carcinoma, a disfiguring disease, is the fifth most frequent cancer worldwide. India has recorded the highest incidence for oral cancer where it accounts for 40-50% of all cancers and most common cancer in both males and females (Moore *et al*., 2000). Oral carcinogenesis occurs after exposure of the entire epithelial surface to the repeated insult of carcinogens (Wang *et al*., 2004). DMBA, a potent organ specific carcinogen is employed to induce oral carcinoma in hamsters buccal pouches since the oral tumors induced by DMBA is morphologically and histologically similar to that of human oral tumors (Miyata *et al*., 2001).

In mammals, the day night rhythmicity of biochemical and physiological processes are regulated by the internal body clock located in the suprachiasmatic nucleus (SCN) of the hypothalamus (Schibler and Sassone Corsi, 2002). Abnormal cellular proliferation is associated with irregular or abnormal functioning of the 24 h circadian rhythm (Kolanjiappan and Manoharan, 2005). It has been reported that the control of endogenous body clock and the control of genes related to initiation and progression of tumors are tightly linked (Fu *et al*., 2002). Amplitude damping, phase shifts and changes in mesor values have been documented in carcinogenesis (Wille, 2003).

Elevated lipidperoxidation and insufficient antioxidant potential has been implicated in the pathogenesis of number of disorders including cancer (Kolanjiappan *et al*., 2003). Recent studies suggested that circadian dysregulation of lipid peroxidation and antioxidants may act as a risk factor for carcinogenesis. Enzymatic and non-enzymatic antioxidants, which control the production of reactive oxygen species, have been repeatedly shown to exhibit circadian rhythmicity (Singh *et al*., 2003). Previous reports from our laboratory demonstrated an altered temporal pattern of lipid peroxides and antioxidants in plasma and erythrocytes of oral cancer patients (Albert Baskar *et al*., 2004; Manoharan *et al*., 2005). However, circadian studies on experimental model will provide an exact reference values for any biochemical variables since the blood samples are simultaneously collected from control and diseased subjects. Investigation of mechanisms by which the circadian clock controls cell proliferation with reference to TBARS and antioxidants status might lead to new therapeutic targets. Thus, the present study was designed to monitor the temporal patterns of blood lipid peroxidation and antioxidants in DMBA induced hamster buccal pouch carcinogenesis.

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MATERIALS AND METHODS

Chemicals: The carcinogen 7,12 dimethylbenz(a) anthracene (DMBA) was obtained from Sigma Aldrich Chemicals Pvt. Ltd., Bangalore, India. All other chemicals used were of analytical grade.

Animals: Male golden Syrian hamsters 8-10 weeks old, weighing 80-120 g, were purchased from National Institute of Nutrition, Hyderabad, India and were maintained in Central Animal House, Rajah Muthaiah Medical College and Hospital, Annamalai University. The animals were housed five in a polypropylene cage and provided standard pellet diet and water *ad libitum*. The animals were maintained under controlled conditions of temperature and humidity with a 12 h light/dark cycles.

Experimental protocol: The experimental design was approved by local institutional ethics committee, Annamalai University, Annamalai Nagar. This experimental study was conducted in the Department of Biochemistry, Annamalai University during the period of January to July 2005.

A total number of 20 hamsters were divided into 2 groups of 10 animals each. Oral cancer was induced in group I animals by painting with 0.5% DMBA in liquid paraffin on the left buccal pouches thrice a week for 14 weeks. All the animals in group I developed oral squamous cell carcinoma at the end of the experimental period. Group II animals served as controls and were painted with liquid paraffin alone. Blood samples were collected from both the oral cancer animals and control animals at 4 h intervals (00:00, 04:00, 08:00, 12:00, 16:00, 20:00, 24:00) continuously throughout the 24 h period. Minimum amount of blood was collected by sinocular puncture into heparinized tubes. The plasma was separated by centrifugation at 3000 rpm for 15 min. After plasma separation, the buffy coat was removed and the packed cells were washed thrice with physiological saline. A known volume of erythrocytes was lysed with 0.2 M hypotonic phosphate buffer (pH 7.4). The hemolyste was separated by centrifugation at 10,000 rpm for 15 min at 20°C. The erythrocyte membrane was prepared according to the method of Dodge *et al.* (1968) modified by Quist (1980).

Biochemical assays: The biochemical estimations were done immediately after blood collection. Thiobarbituric acid reactive substances in plasma was assayed by the method of Yagi (1978) and in erythrocyte membranes by the method of Donnan (1950). The activities of SOD, CAT and GPx in erythrocyte lysate were estimated according to the methods of Kakkar *et al.* (1984), Sinha (1972) and Rotruck *et al.* (1973), respectively.

Statistical analysis: The Cosinorwin computer software program was used to analyse circadian characteristics such as mesor (a rhythm adjusted mean value, usually more accurate than the arithmetic mean), circadian amplitude (a measure of half of the difference between maximum and minimum rhythmic variability in a cycle), the circadian acrophase (peak time of the variable) and r-value (correlation coefficient). The mean values of the biochemical variables (mean±SD) were plotted (Y-axis) against the time of blood collection (X-axis).

RESULTS AND DISCUSSION

Table 1 and 2 show the characteristics of circadian patterns (acrophase, amplitude, mesor etc.,) of plasma and erythrocytes TBARS and enzymatic antioxidants (SOD, CAT and Gpx), respectively in control animals and hamsters with buccal pouch carcinogenesis. The acrophase of TBARS and enzymatic antioxidants were delayed in DMBA induced oral carcinogensis. The mesor values were significantly increased for TBARS whereas decreased for enzymatic antioxidants in plasma and erythrocytes of cancer animals as compared to control

Table 1: Circadian characteristics of plasma and erythrocyte membrane TBARS in control and oral cancer animals

Parameters	Circadian characteristics	Control animals	Oral cancer animals
Plasma TBARS	Acrophase	16.39	22:33
	Mesor	2.8	3.8
	Amplitude	1.2	0.6
	r-value	0.52 [dr] ($p<0.20$)	0.32 [nsr] ($p<0.50$)
Erythrocyte membrane TBARS	Acrophase	17:50	21:38
	Mesor	0.3	1.2
	Amplitude	0.1	0.2
	r-value	0.77 [dr] ($p<0.10$)	0.23 [nsr] ($p<0.50$)

dr; detectable rhythmicity, nsr; no significant rhythmicity, r; correlation coefficient

Table 2: Circadian characteristics of SOD, CAT and GPx activities in erythrocytes of control and oral cancer animals

Parameters	Circadian characteristics	Control animals	Oral cancer animals
SOD	Acrophase	14:10	17:17
	Mesor	2.3	1.7
	Amplitude	0.8	0.2
	r-value	0.51 [dr] ($p<0.20$)	0.27 [nsr] ($p<0.50$)
CAT	Acrophase	13:20	16:30
	Mesor	1.9	1.3
	Amplitude	0.5	0.3
	r-value	0.52 [dr] ($p<0.20$)	0.37 [nsr] ($p<0.50$)
GPx	Acrophase	13:36	16:39
	Mesor	20.1	12.1
	Amplitude	1.3	1.1
	r-value	0.62 [dr] ($p<0.10$)	0.36 [nsr] ($p<0.50$)

dr; detectable rhythmicity, nsr; no significant rhythmicity, r; correlation coefficient

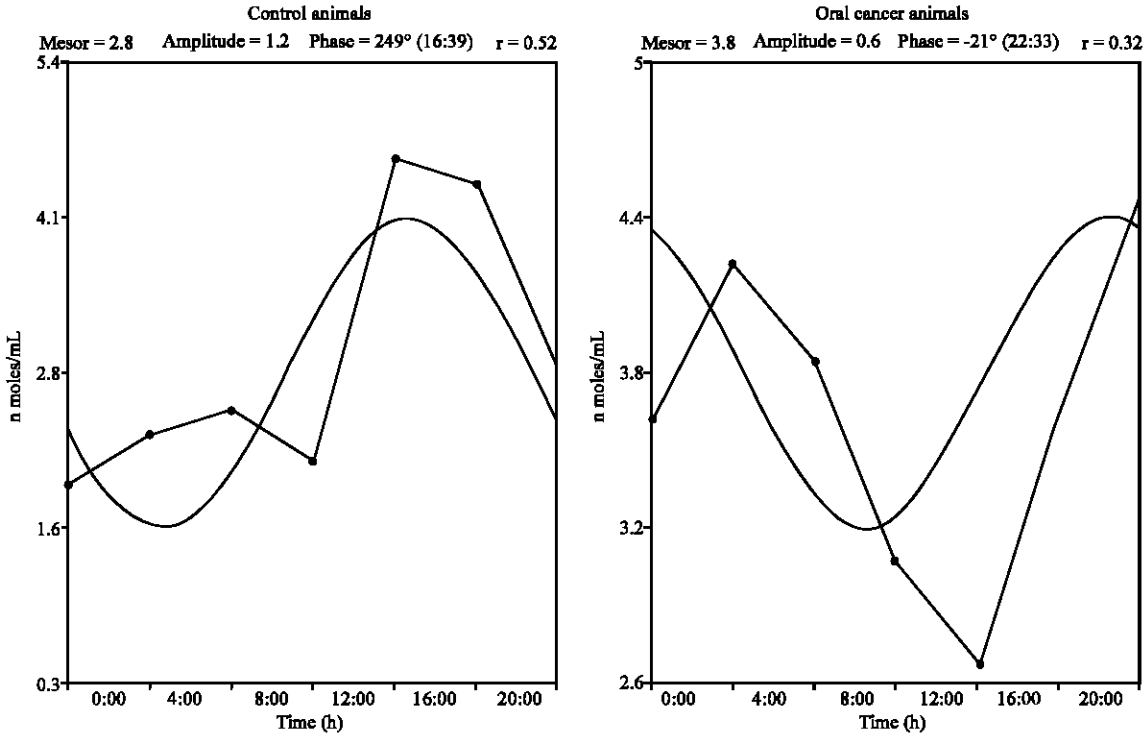

Fig. 1: Temporal patterns of plasma TBARS in control and oral cancer animals

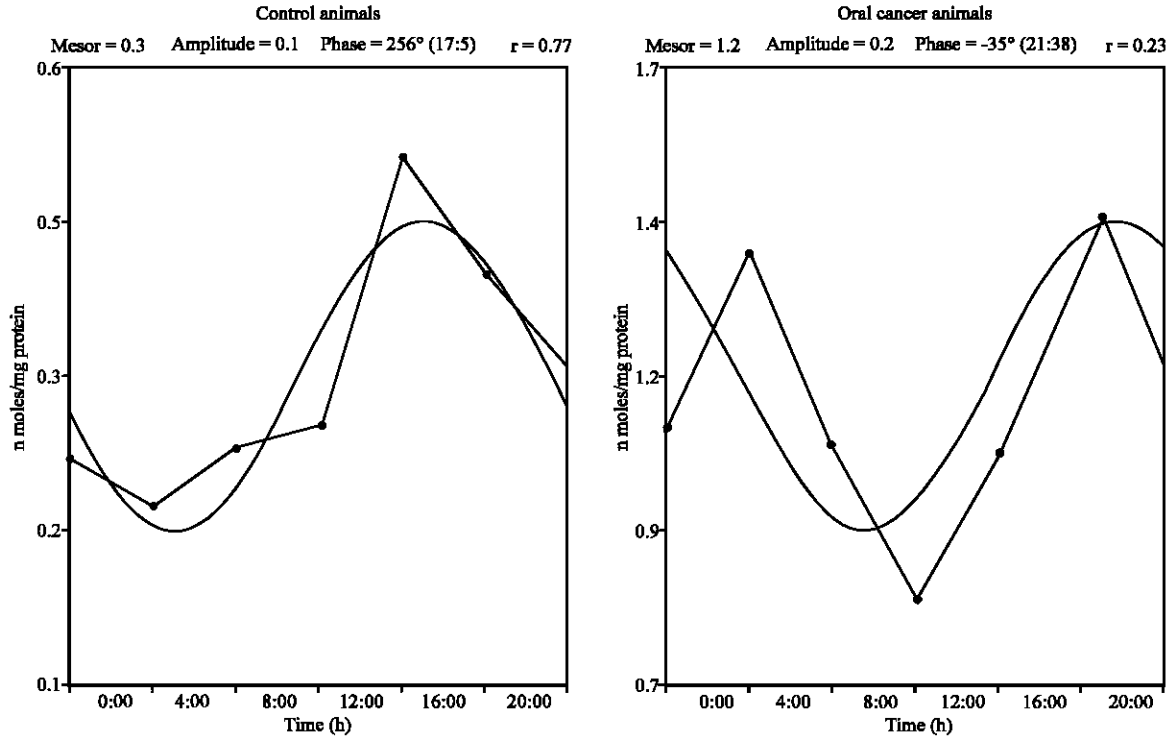

Fig. 2: Temporal patterns of erythrocyte membrane TBARS in control and oral cancer animals

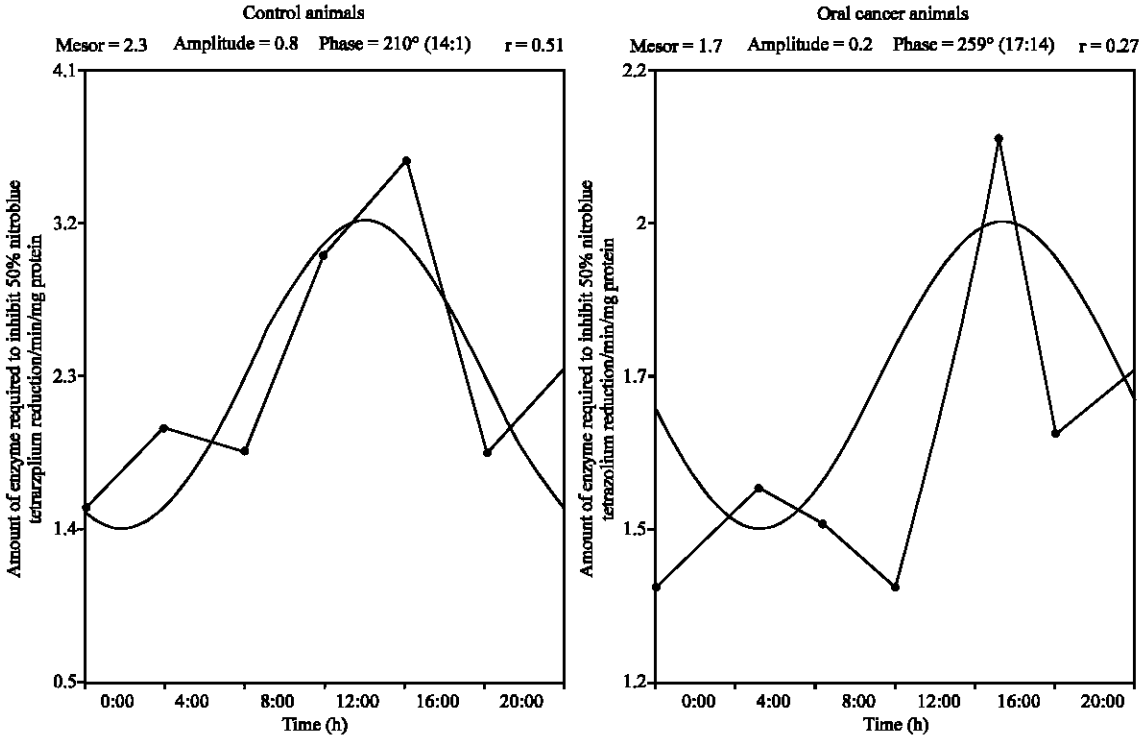

Fig. 3: Temporal patterns of erythrocyte superoxide dismutase activity in control and oral cancer animals

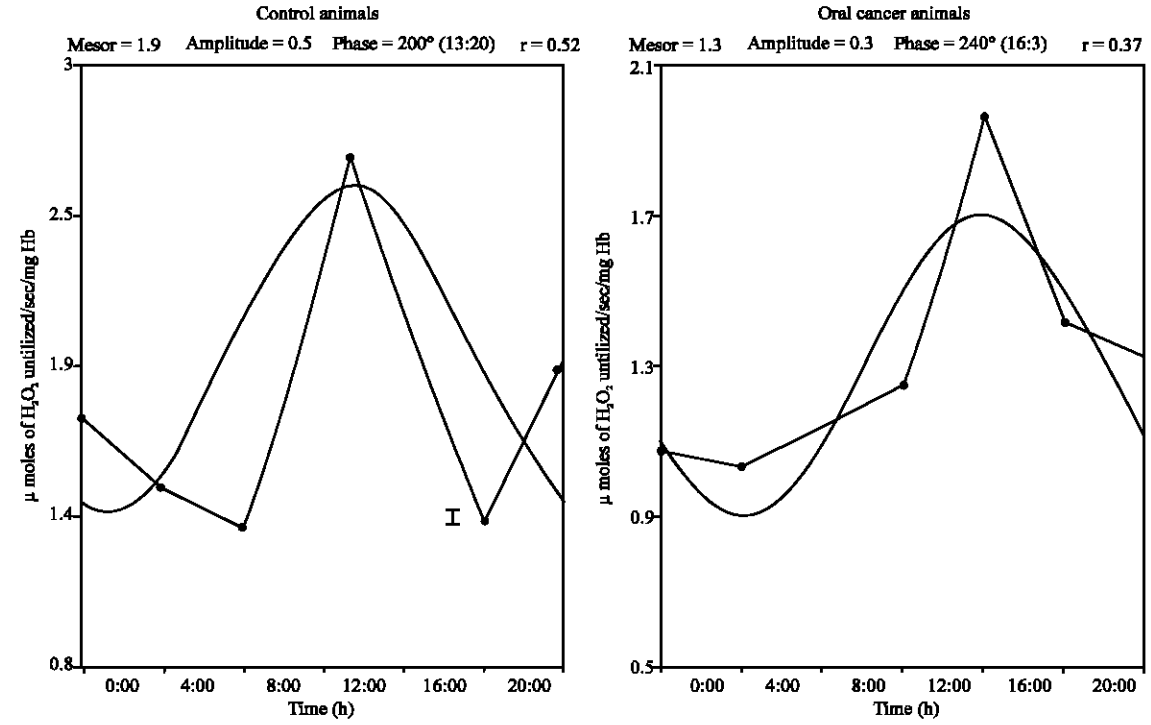

Fig. 4: Temporal patterns of erythrocyte catalase activity in control and oral cancer animals

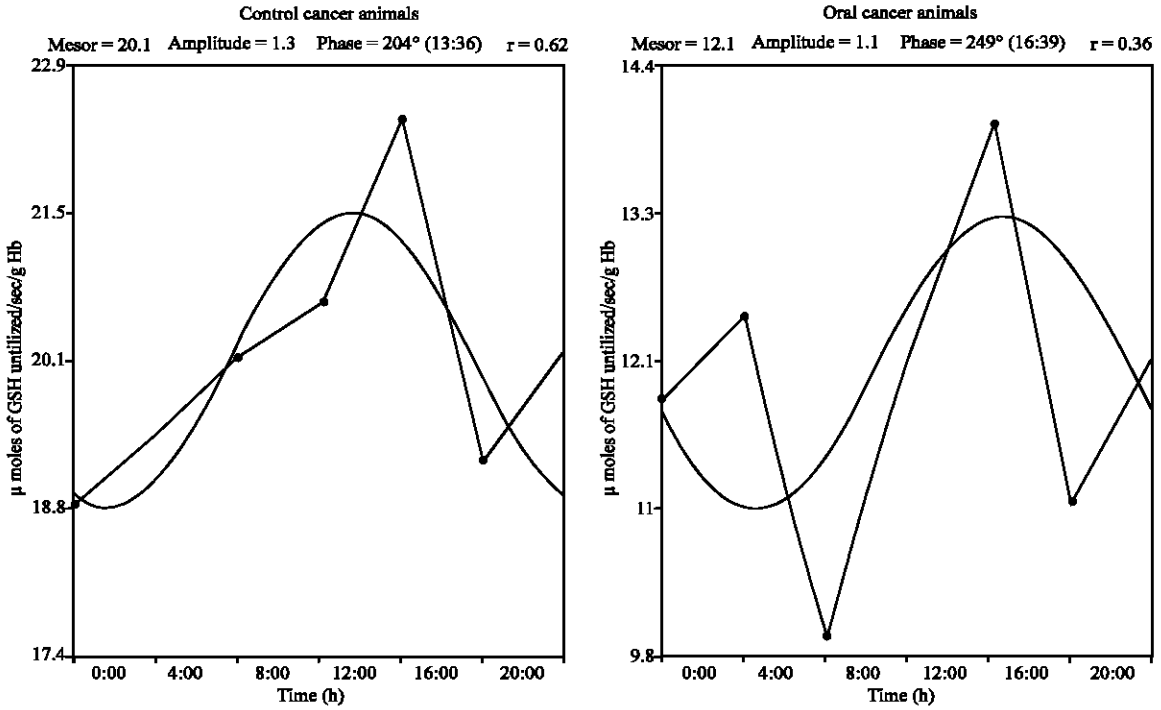

Fig. 5: Temporal patterns of erythrocyte glutathione peroxidase activity in control and oral cancer animals

animals. Cosinorwin analysis revealed a detectable rhythmicity in the control animals and an altered rhythms in cancer animals (Fig. 1-5).

In recent years, several scientists focused their attention in the role of free radicals and antioxidants at the tissue level in the causation of cancer. Disrupted circadian rhythm due to overproduction of reactive oxygen species and decline in antioxidants has been well documented in animal and human cancer models (Sephton and Spiegel, 2003; Levi, 2002). The chronobiological approach on the role of reactive oxygen species and antioxidants in cancer processes might lead to new chronotherapeutic agents (Singh *et al*., 2003).

In the present study, the diurnal rhythm of TBARS and antioxidants exhibited marked phase shift over a 24 h period. The thiobarbituric acid reactive substances peaks at 16:39 h (plasma) and 17:50 h (erythrocyte membrane) in control animal whereas peaks at 22:33 h (plasma) and 21:36 h (erythrocyte membranes) in hamsters with buccal pouch carcinogenesis. A 4-6 h phase delay was noticed for TBARS in cancer animals as compared to control animals. Furthermore a significant increase in 24 h mean values (mesor) for TBARS in cancer animals indicate that the erythrocytes of tumor bearing animals are more susceptible to oxidative damage. Highest plasma and erythrocytes TBARS were reported during night time in cancer patients and cancer animals (Akbulut *et al*., 2003; Kolanjiappan and Manoharan, 2005). Our results lendcredibility to these suggestions.

A statistically significant circadian rhythm and altered rhythms were recorded in SOD, CAT and GPx activities in control and experimental animals, respectively. The activities of enzymatic antioxidants were peak at 14:10 h (SOD), 13:20 h (CAT) and 13:36 h (GPx) in control animals. The acrophase was delayed ~3 h for enzymatic antioxidant activities in tumor bearing animals. (Kolanjiappan and Manoharan, 2005; Manivasagam and Subramanian, 2004) have reported that antioxidant enzyme activities were higher during the light than during dark span in experimental rats. Our results corroborate these observations.

The mesor (24 h mean value) for enzymatic antioxidants (SOD, CAT and GPx) were delayed markedly in cancer animals as compared to control animals. Lowered enzymatic antioxidants activities have been reported in several cancers including oral carcinoma (Zhao *et al*., 2005; Supapriya *et al*., 2002). The declined activities of enzymatic antioxidants in DMBA treated animals as reflected by decreased mesor values could be due to exhaustion of these enzymatic antioxidants to scavenge the excess lipid peroxidation byproducts. The alteration in the acrophase of TBARS rhythms in cancer animals may therefore be correlated to the altered diurnal rhythmicity in activities of enzymatic antioxidants. The

present study therefore demands further investigation to understand the mechanism on the regulation of TBARS and antioxidants by circadian pacemaker, which may help for the chronotherapeutic efficacy in carcinogenesis.

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