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Sleep pattern and locomotor activity are impaired by doxorubicin in non-tumor-bearing rats☆

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ABSTRACT

Purpose: We sought explore the effects of doxorubicin on sleep patterns and locomotor activity. To investigate these effects, two groups were formed: a control group and a Doxorubicin (DOXO) group.
Methods: Sixteen rats were randomly assigned to either the control or DOXO groups. The sleep patterns were examined by polysomnographic recording and locomotor activity was evaluated in an open-field test.
Results: In the light period, the total sleep time and slow wave sleep were decreased, while the wake after sleep onset and arousal were increased in the DOXO group compared with the control group ($p < 0.05$). In the dark period, the total sleep time, arousal, and slow wave sleep were increased, while the wake after sleep onset was decreased in the DOXO group compared with the control group ($p < 0.05$). Moreover, DOXO induced a decrease of crossing and rearing numbers when compared control group ($p < 0.05$).
Conclusions: Therefore, our results suggest that doxorubicin induces sleep pattern impairments and reduction of locomotor activity.

1. Introduction

Although sleep is essential for good health and quality of life, according to Bonnet and Arand [1], one-third or more of normal adults suffer from significant sleep loss. In addition, several studies have shown that cancer chemotherapy treatment with doxorubicin alters sleep patterns and health status, leading to distressing symptoms and fatigue [2–4].

Doxorubicin (DOXO), a member of the antineoplastic antibiotic family of anthracyclines, is a chemotherapeutic agent developed in the 1960s [5], that is still widely used in the treatment of a variety of malignancies, such as acute leukemia, non-Hodgkin lymphomas, breast cancer, Hodgkin's disease, and sarcomas [6,7].

Savard et al. [4] showed that breast cancer patients treated with doxorubicin have impaired sleep-wake activity rhythms. Moreover, the first administration of chemotherapy is associated with a disruption of the sleep-wake rhythm, and the repeated administration of this chemotherapy results in more enduring impairments of the sleep-wake rhythms. Moreover, the DOXO treatment in breast cancer women is associated with disturbance sleep, sleep efficiency and poor sleep quality [8].

Neural systems implicated in the control of sleep also impact the functioning of host defenses. The challenge for future research is to determine the ultimate implication of the sleep loss effects in molecular terms to clarify the mechanistic processes involved in the impairment of cellular functional activity, and the impact of sleep deprivation on

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other essential inflammatory markers for immune function [9]. On the other hand, other studies have reported that chemotherapy may promote and/or aggravate inflammation status which impairs sleep quality [10,11].

Therefore, in this study, we sought to determine effects of DOXO on the sleep pattern and locomotor activity in the rats.

2. Methods

2.1. Animals

The Experimental Research Committee of the Universidade Federal de São Paulo approved all procedures for the care of the animals used in this study (0619/09). A total of 16 male Wistar rats approximately 14 weeks of age (weighing 350–380 g) were used. They were housed four per cage in an animal room under a 12-h light-dark cycle at 22 ± 1 °C and 60 ± 5% humidity and received a chow diet and water ad libitum. The experiments were carried out after a one-week acclimation period. The rats were randomly divided into two groups: (i) a saline Control group (n=8) and (ii) a doxorubicin (DOXO) group (n=8).

2.2. Design

In the first day the animal were subjected to electrode insertion surgery. Seven days after surgery (ninth day), the animal received doxorubicin cloridrato (Eurofarma Laboratory, Campinas, Brazil) (15 mg/kg, i.p.) [12] or saline (i.p.). The sleep recording was conducted for 24 h (12-h light-dark) after 48 h doxorubicin-treatment (a single administration) and the locomotor activity was evaluated 48 h after doxorubicin-treatment in the open-field test. The doxorubicin and saline were administered at 7:00 AM.

2.3. Experimental Protocols

2.3.1. Surgical preparation

The rats were anesthetized with diazepam and ketamine (5 and 100 mg/kg body weight, i.p., respectively). They were then placed in the stereotaxic apparatus, and two bipolar electrodes with 4 stainless-steel screws (Ø 0.9 mm) were placed into the skull through small holes bored into the right lateral frontoparietal region (1 pair of screws) and the left medial frontoparietal region (another pair) in order to monitor the bipolar electrocorticogram (ECoG) [13].

For the electromyography recording (EMG), one pair of electrodes was inserted in the cervical musculature. After the electrode insertion surgery, the rats were placed in individual compartments for 7 days of recovery and then given 2 days of adaptation while connected to the polysomnographic recording (PSG) device.

2.3.2. Electrocorticography recording

During the Electrocorticography (ECoG) recording, the rats remained in individual compartments with unrestricted access to food and water. The ECoG recording was made with a Nihon-Kohden model QP 223 polygraph (digital signal acquisition) using three pairs of channels: two ECoG and one EMG for the cervical musculature. The recording was analyzed for two 12 h periods (12-h light–dark). In the literature, rats were shown to have 62% sleep efficiency during the light period (7:00–19:00 h) and 33% during the dark period (19:00–7:00 h) [14]. Each 10 s period was classified in accordance with Timo-Iaria et al. [15]: wakefulness (W) was defined as low amplitude waves with fast ECoG and EMG activation; slow wave sleep (SWS) was defined as high amplitude waves and slow ECoG and EMG activation; and paradoxical sleep was defined (PS) as fast ECoG activity, the regular presence of a theta hippocampal rhythm and the absence of EMG activity. At the end of the analysis, the sleep parameters were quantified using the Polysmith Software program®.

The sleep parameters collected were the following: sleep efficiency (SE; percentage of total sleep time during the recording period), latency to sleep (time lag between the start of the recording and the first sleep period), slow wave sleep (SWS; percentage of all periods featuring high delta content during the recording period), paradoxical sleep (PS; percentage of all periods during the recording period), PS latency (time lag between the start of the recording and the first PS period), wake after sleep onset (WASO; percentage of all periods of wakefulness throughout the recording period number of awakenings) and number of arousals (number of awakenings).

2.3.3. Open-field test

The rats were treated with doxorubicin (15 mg/kg), and saline 48 h before the exposure to the open-field apparatus (light phase), in order to assess the possible effects of drug treatment on spontaneous locomotor activity. Analysis of the rat's spontaneous activity was carried out in an open field apparatus, which is a 45 cm×60 cm arena surrounded by 50 cm high walls made of brown plywood with a frontal glass wall. The floor of the open field was divided into 9 rectangles (15 cm×20 cm each) by black lines [16]. Animals were gently placed on the left rear quadrant, and left to explore the arena for 5 min. The number of horizontal (crossings) and vertical (rearings) activities performed by each rat during the 5 min observation period was counted by an expert observer.

2.4. Statistical analysis

The statistical analysis was performed using the GraphPad Prism statistics software package version 5.0 for Windows (GraphPad Software, San Diego, CA, USA). The data are expressed as the mean ± SEM. Implementation of the Kolmogorov-Smirnov test revealed that the results of the experiments were distributed normally. The data were analyzed using two-way ANOVA followed by the Tukey test and unpaired Student's *t*-test for comparison between the two groups. A value of $P < 0.05$ was considered statistically significant.

3. Results

3.1. Doxorubicin administration impairs the sleep pattern

A significant increase was detected on the wakefulness of the DOXO group in light period in relation to control group. Statistical differences were found in both the light/dark period in the DOXO and control groups for wakefulness. In addition, the Sleep Efficiency of the DOXO group demonstrated a significant decrease in relation to the control group in the light period group; no statistical differences were found in either the light/dark period in the DOXO group to Sleep Efficiency. Fig. 1.

The sleep parameter data are shown in Table 1. The two-way ANOVA revealed the main effects of the group (arousal), time (TST,

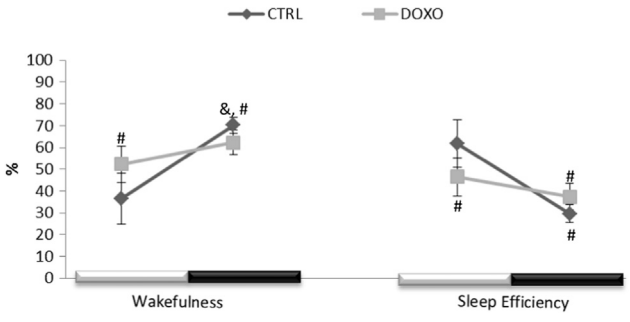

Fig. 1. Wakefulness (%) and Sleep Efficiency (%) during light and dark periods of sleep in both Control (CTRL) and Doxorubicin (DOXO) groups. Two-way ANOVA followed by the Tukey post hoc test ($p < 0.05$) comparison of groups for the time factor (# differ Light Control; & differ Light DOXO). The bars mark of periods light (left) and dark (right) of sleep. Animals: CTRL (n=8), DOXO (n=8). Dose: 15 mg/kg, (i.p.) – DOXO or saline.

Table 1
Sleep pattern in Control and Doxorubicin groups during the light and dark periods (12-h light–dark).

Sleep pattern	Control		DOXO		ANOVA F		
	Light	Dark	Light	Dark	Time	Group	Interaction
Total Sleep Time (min)	443.35 ± 78.85*	212.75 ± 28.96	332.44 ± 63.39*#	268.98 ± 44.37*#	29.811[a]	1.030	9.63[c]
Wake after Sleep Onset (min)	256.22 ± 88.31	500.17 ± 24.76	362.72 ± 57.26*#	442.80 ± 33.65*#	38.083[a]	0.875	9.740[c]
Latency Sleep (min)	17.97 ± 32.10	6.80 ± 11.36	20.58 ± 15.25	6.18 ± 9.50	2.132	0.012	0.033
Arousal	78.50 ± 12.66	44.50 ± 14.79	121.80 ± 26.19*#	104.80 ± 33.70*	4.802[a]	19.815[b]	0.533
PS Latency (min)	64.75 ± 82,03	24.45 ± 22.34	47.28 ± 31.80	55.50 ± 82.16	0.303	0.054	0.694
Slow Wave Sleep (%)	54.42 ± 9.28*	26.80 ± 3.54	40.56 ± 9.01*#	34.96 ± 6.10*#	22.288	0.657	9.794[c]
Paradoxal sleep (%)	9.15 ± 2.71*	2.97 ± 0.37	7.22 ± 1.45*	2.74 ± 1.21*#	47.836	1.975	1.210

P≤0.05 comparing groups for the [a]time factor, [b]group factor and [c]interaction (two-way ANOVA followed by the Tukey test). # differ Light Control; * differ Dark Control; & differ Light DOXO. Animals: CTRL (n=8), DOXO (n=8). Dose: 15 mg/kg, (i.p.) – DOXO or saline.

WASO and arousal) as well as an interactive effect between these factors (TST, WASO and SWS). During the light period, the total sleep time (min) and slow wave sleep (%) were decreased, while the wake after sleep onset (min) and arousal were increased in the DOXO group compared with the control group (light period) ($p < 0.05$). In contrast, during the dark period, the total sleep time (min), arousal and slow wave sleep (%) were increased, while the wake after sleep onset (min) was decreased in the DOXO group compared with the control group (dark period) ($p < 0.05$).

3.2. Doxorubicin leads to decreased locomotor activity when evaluated by open field test

The results depicted in Fig. 2A show the effect of rats treated with Doxorubicin or vehicle on the locomotor activity in the open field test. T-test revealed significant differences for control and DOXO groups ($t(19): 7.72$; $p < 0.01$), indicating that doxorubicin induced a reduction of locomotor activity. The Fig. 2B indicated that doxorubicin induced low exploratory activity. T-test revealed significant differences for control and DOXO group ($t(19): 6.61$; $p < 0.01$). The Open Field Test was performed for 48 h and 72 h (data not shown) after DOXO. However, the results were the same, thus we chose the results from the 48 h (OFT) test to assess the animals' locomotor activity before evaluating their sleep patterns.

4. Discussion

The purpose of the present study was to examine the effect of a single administration of DOXO (15 mg/kg) on sleep-wake cycles and locomotor activity. This study demonstrated that a single administration of DOXO had impaired the sleep pattern and reduced the locomotor activity of rats.

These changes are relevant because these alterations negatively impact the quality of life of chemotherapy patients, leading to higher levels of fatigue [17]. Borniger et al. [18] demonstrated in adult female c57bl/6 mice that 13.5 mg/kg doxorubicin and 135 mg/kg cyclophosphamide increased Non Rapid Eye Movement (NREM) and Rapid Eye Movement (REM) sleep during subsequent active (dark) phases; this induced sleep was fragmented and of low quality. Similarly, our results demonstrated an increased in NREM/SWS (dark phase) and fragmented alterations with DOXO treatment, being that the fragmentation can be seen in increased arousal index of DOXO group; i.e. how often the animal came into wakefulness per hour (DOXO group – Light phase: 21.98 arousal/hour; Dark phase: 23.39 arousal/hour versus Control group – Light phase: 10.63 arousal/hour; Dark phase: 12.57 arousal/hour).

In the present study, the rats presented disrupted circadian rhythms after DOXO treatment. Sleep efficiency showed no statistical differences in either the light or dark periods, thus, the light and dark period presented similar sleep efficiency levels. This is contrary to the pattern reported by Van Luijtelarr and Coenen [14] which presented 62% predominance of sleep in the light period and 33% in the active or dark period.

Last year, it was recognized that DOXO reaches the brain capillary endothelial cells by a P-glycoprotein, leading to attenuated blood-brain barrier (BBB) permeation. Likewise, P-glycoprotein inhibitors at the BBB may increase drug concentrations in the central nervous system [19,20]. Likewise, several studies have reported that chemotherapeutics, such as DOXO, activate the immune system with an increased inflammatory cytokine release in both central and peripheral tissues which induces sleep problems [10,11]. This peripheral-CNS axis occurs by transport of cytokines to the brain via the vagus nerve [21]. This likely explains the role of pro-inflammatory cytokines in vigilance state. Hogan and collaborators injected IL6 indirectly into the CNS from rodents and found enhanced sleep fragmentation [22]. Moreover, it was observed in women diagnosed with stage I–III breast cancer

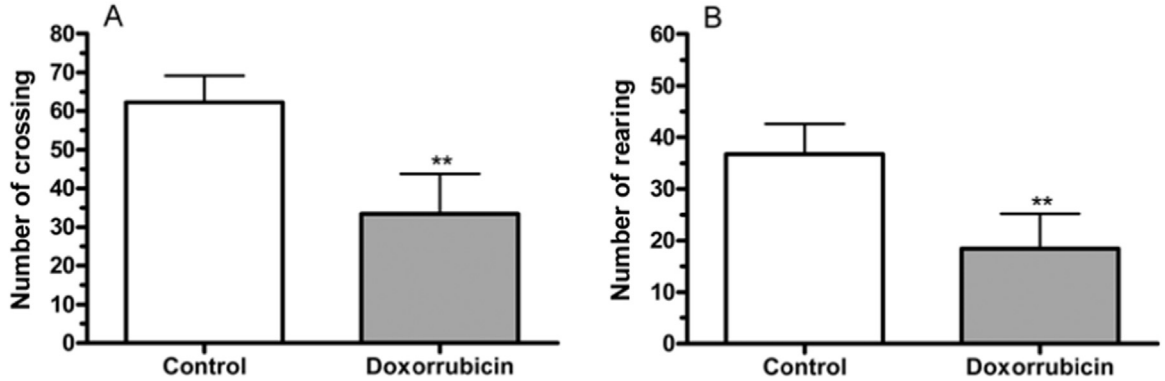

Fig. 2. Effect of treatment with doxorubicin on locomotor activity evaluated in the open-field test. The results are shown as the number of crossings (panel 1A) and the number of rearing (panel 1B). The results are expressed as the mean ± MSD (n=9–12). **$p < 0.01$ when compared to control group. Animals: CTRL (n=8), DOXO (n=8). Dose: 15 mg/kg, (i.p.) – DOXO or saline.

receiving chemotherapy, that blood IL6, C-reactive protein and IL1ra concentrations are associated with sleep fragmentation [23]. Recently, a correlation between IL6 mRNA expression and disruption sleep in health rats was identified [18].

Another important consideration in our study is the apparent decrease in locomotor activity and exploratory behavior observed in the rats after 48 h of DOXO intraperitoneal treatment. The results of the present work demonstrated that DOXO reduced the crossing and rearing number in the open field test. A study performed by Liedke et al. [24] showed that animals treated with DOXO (0.5, 2 and 8 mg/kg), evaluated in the open field 20 min after DOXO, presented no differences in anxiety, locomotor activity and exploratory behavior. On the other hand, when the animals were tested 24 h after DOXO treatment a reduced rearing number was observed, suggesting that DOXO can interfere with exploratory behavior. Several studies have previously shown that sleep alterations and sleep-deprived rats exhibit body weight loss and elevated corticosterone levels [25–27]. The immediate purpose of glucocorticoid release during stress situations is to provide readily usable energy (i.e., glucose to the central nervous system). This release is accomplished together with catecholamines, GH and glucagon, to induce lipolysis, glycogenolysis, gluconeogenesis and, in cases of severe and prolonged stress, proteolysis [28]. These alterations can play an important role in the onset and maintenance of wasting found in the DOXO treatment. A study performed by Kaur et al. [29] showed that rats subjected to restraint stress demonstrated a reduction of locomotor and exploratory activity. Liu et al. [30] showed similar results in the chronic, unpredicted mild stress in rats. Moreover, stress is involved with the increase of corticosterone level as well reduction of locomotor activity and exploratory behavior in rats [31]. These results indicated that DOXO appears to induce a stress-like state. Recently, de Lima Junior [32] has showed that DOXO leads to conditions similar to cachexia, with severe glucose intolerance both in vivo and in vitro. We suggest that DOXO promotes less muscle mass, strength, and function, supporting the decrease in locomotor activity. However, this hypothesis is speculative and, therefore, further studies are needed.

A point to note in the study was the dose of Doxo used in the procedure. This is not really used in clinical practice, however, this dose is currently used in several studies for analyzed of acute toxicity in the characterization of the molecular pathways in cardiotoxicity, sarcopenia and autophagy in skeletal muscle [33–35].

In this context, our results suggest that doxorubicin induces sleep pattern impairment that is accompanied by locomotor activity alterations. Thus, although it is effective in promoting tumor cell death, this chemotherapy causes severe damage to the sleep and locomotor behavior decreasing patient quality of life.

Conflict of Interest statement missing

The authors declare that no conflicts of interest exist.

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References

[1] Bonnet MH, Arand DL. We are chronically sleep deprived. Sleep 1995;18:908–11.
[2] Payne LC, Obal FJr, Krueger JM. Hypothalamic releasing hormones mediating the effects of interleukin-1 on sleep. Cell Biochem 1993;53:309–13.
[3] Berger AM, Higginbotham P. Correlates of fatigue during and following adjuvant breast cancer chemotherapy: a pilot study. Oncol Nurs Forum 2000;27:1443–8.
[4] Savard J, Liu L, Natarajan L, Rissling MB, Neikrug AB, He F, et al. Breast cancer patients have progressively impaired sleep-wake activity rhythms during chemotherapy. Sleep 2009;32:1155–60.
[5] Leonard RC, Williams S, Tulpule A, Levine AM, Oliveros S. Improving the therapeutic index of anthracycline chemotherapy: focus on liposomal doxorubicin (Myocet). Breast 2009;18(4):218–24.
[6] Young RC, Ozols RF, Myers CE. The anthracycline antineoplastic drugs. N Engl J Med 1981;305(3):139–53.
[7] Lai HC, Yeh YC, Ting CT, Lee WL, Lee HW, Wang LC, et al. Doxycycline suppresses doxorubicin-induced oxidative stress and cellular apoptosis in mouse hearts. Eur J Pharm 2010;644(1–3):176–87.
[8] Sanford SD, Wagner LI, Beaumont JL, Butt Z, Sweet JJ, Cella D. Longitudinal prospective assessment of sleep quality: before, during, and after adjuvant chemotherapy for breast cancer. Support Care Cancer 2013;21:959–67.
[9] Irwin MR, Carrillo C, Olmstead R. Sleep loss activates cellular markers of inflammation: sex differences. Brain Behav Immun 2010;24(1):54–7.
[10] Mills PJ, Parker B, Dimsdale JE, Sadler GR, Ancoli-Israel S. The relationship between fatigue and quality of life and inflammation during anthracycline-based chemotherapy in breast cancer. Biol Psychiatry 2005;69:85–96.
[11] Janelsins MC, Mustian KM, Palesh OG, Mohile SG, Peppone LJ, Sprod LK, et al. Differential expression of cytokines in breast cancer patients receiving different chemotherapies: implications for cognitive impairment research. Support Care Cancer 2012;20:831–9.
[12] Santos RV, MLJr Batista, Caperuto EC, Costa Rosa LF. Chronic supplementation of creatine and vitamins C and E increases survival and improves biochemical parameters after doxorubicin treatment in rats. Clin Exp Pharm Physiol 2007;34(12):1294–9.
[13] Rechtschaffen A, Bergmann BN, Everson CA, Kushida CA, Gllilland MA. Sleep deprivation rat. Sleep 1989;12:5–12.
[14] van Luijtelaar EL, Coenen AM. An EEG averaging technique for automated sleep–wake stage identification in the rat. Physiol Behav 1984;33:837–41.
[15] Timo-Iaria C, Negrao N, Schmidek WR, Hoshino K, Lobato de Menezes CE, Leme da Rocha T. Phases and states of sleep in the rat. Physiol Behav 1970;5:1057–62.
[16] Frey JM, Morgan WW, Ticku MK, Huffman RD. A dietary haloperidol regimen for inducing dopamine receptor supersensitivity in rats. Pharm Biochem Behav 1987;26(4):661–9.
[17] Mormont MC, Waterhouse J. Contribution of the rest-activity circadian rhythm to quality of life in cancer patients. Chronobiol Int 2002;19:313–23.
[18] Borniger JC, Gaudier-Diaz MM, Zhang N, Nelson RJ, DeVries AC. Cytotoxic chemotherapy increases sleep and sleep fragmentation in non-tumor-bearing mice. Brain Behav Immun 2015;47:218–27.
[19] Zhao YL, Du J, Kanazawa H, Sugawara A, Takagi K, Kitaichi K, et al. Effect of endotoxin on doxorubicin transport across blood-brain barrier and P-glycoprotein function in mice. Eur J Pharm 2002;2445(1–2):115–23.
[20] Deeken JF, Löscher W. The blood-brain barrier and cancer: transporters, treatment, and trojan horses. Clin Cancer Res 2007;13(6):1663–74.
[21] Ahles TA, Saykin AJ. Candidate mechanisms for chemotherapy-induced cognitive changes. Nat Rev Cancer 2007;7(3):192–201.
[22] Hogan D, Morrow JD, Smith EM, Opp MR. Interleukin-6 alters sleep of rats. J Neuroimmunol 2003;137:59–66.
[23] Liu L, Mills PJ, Rissling M, Fiorentino L, Natarajan L, Dimsdale JE, et al. Fatigue and sleep quality are associated with changes in inflammatory markers in breast cancer patients undergoing chemotherapy. Brain Behav Immun 2012;26:706–13.
[24] Liedke PE, Reolon GK, Kilpp B, Brunetto AL, Roesler R, Schwartsmann G. Systemic administration of doxorubicin impairs aversively motivated memory in rats. Pharm Biochem Behav 2009;94(2):239–43.
[25] Esumi LA, Palma BD, Gomes VL, Tufik S, Hipolide DC. Inflammatory markers are associated with inhibitory avoidance memory deficit induced by sleep deprivation in rats. Behav Brain Res 2011;221:7–12.
[26] Hipólide DC, Suchecki D, Pimentel de Carvalho Pinto A, Chiconelli Faria E, Tufik S, Luz J. Paradoxical sleep deprivation and sleep recovery: effects on the hypothalamic-pituitary-adrenal axis activity, energy balance and body composition of rats. J Neuroendocr 2006;18(4):231–8.
[27] Rosa Neto JC, Lira FS, Venancio DP, Cunha CA, Oyama LM, Pimentel GD, et al. Sleep deprivation affects inflammatory marker expression in adipose tissue. Lipids Health Dis 2010;30(9):125.
[28] Sapolsky RM, Romero LM, Munck AU. How do glucocorticoids influence stress responses? Integrating permissive, suppressive, stimulatory, and preparative actions. Endocr Rev 2000;21:55–89.
[29] Kaur R, Jaggi AS, Singh N. Studies on effect of stress preconditioning in restrain stress-induced behavioral alterations. Yakugaku Zasshi 2010;130(2):215–21.
[30] Liu X, Yang le J, Fan SJ, Jiang H, Pan F. Swimming exercise effects on the expression of HSP70 and iNOS in hippocampus and prefrontal cortex in combined stress. Neurosci Lett 2010;476(2):99–103.
[31] Küçük A, Gölgeli A, Saraymen R, Koç N. Effects of age and anxiety on learning and memory. Behav Brain Res 2008;195(1):147–52.
[32] de Lima Junior EA, Yamashita AS, Pimentel GD, De Sousa LG, Santos RV, Gonçalves CL, et al. Doxorubicin caused severe hyperglycaemia and insulin resistance, mediated by inhibition in AMPk signalling in skeletal muscle. J Cachex Sarcopenia Muscle 2016. http://dx.doi.org/10.1002/jcsm.12104, [Epub ahead of print].
[33] Saeed NM, El-Naga RN, El-Bakly WM, Abdel-Rahman HM, Salah ElDin RA, El-Demerdash E. Epigallocatechin-3-gallate pretreatment attenuates doxorubicin-induced cardiotoxicity in rats: a mechanistic study. Biochem Pharmacol 2015;95(3):145–55.
[34] Yu AP, Pei XM, Sin TK, Yip SP, Yung BY, Chan LW, et al. [D-Lys3]-GHRP-6 exhibits pro-autophagic effects on skeletal muscle. Mol Cell Endocrinol 2015;401:155–64.
[35] Dirks-Naylor AJ, Tran NT, Yang S, Mabolo R, Kouzi SA. The effects of acute doxorubicin treatment on proteome lysine acetylation status and apical caspases in skeletal muscle of fasted animals. J Cachex Sarcopenia Muscle 2013;4(3):239–43.