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Sleep deprivation worsened oral ulcers and delayed healing process in an experimental rat model

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ABSTRACT

Aims: Sleep deficiency has been reported to be associated with some oral health problems. Oral ulcers are very common lesions of the oral mucosa, which severely impact patients' quality of life. However, the association between sleep deficiency and the oral ulcer remains unknown. The present study aims to explore the effects of sleep deficiency on oral ulcers.
Main methods: Rats were divided into normal control group (n = 30) and oral ulcer group (OU group, n = 50). Model rats with phenol-induced oral ulcers were deprived of sleep for 72 h by using the modified multiple platform technique.
Key findings: Sleep deprivation worsened oral ulcers and delayed healing process in rats. In addition, sleep deprivation increased the γ-aminobutyric acid (GABA, $P < 0.01$) and 5-hydroxytryptamine (5-HT, $P < 0.05$) levels in serum and brain, the corticotrophin (ACTH, $P < 0.05$), corticosterone (CORT, $P < 0.01$), immunoglobulin (Ig)M ($P < 0.01$), tumor necrosis factor-alpha (TNF-α) ($P < 0.01$), interleukin (IL)-1β ($P < 0.01$), IL-6 ($P < 0.01$), IL-8 ($P < 0.01$), monocyte chemoattractant protein-1 (MCP-1) ($P < 0.01$), and 8-hydroxy-deoxyguanosine (8-OHdG, $P < 0.01$) levels in serum. Sleep deprivation also up-regulated malonaldehyde (MDA) ($P < 0.05$), TNF-α ($P < 0.05$), and IL-1β ($P < 0.01$) levels in oral mucosa tissue and delayed superoxide dismutase (SOD, $P < 0.05$) activity recovery.
Significance: These data suggest that sleep deprivation impaired the oral ulcer healing in rat oral mucosa, and the mechanisms of this effect are probably related to neuro-immuno-endocrine system and oxidative stress.

1. Introduction

Sleep deficiency increasingly characterizes modern society, leading to a wide range of serious health problems. Sleep influences a vast array of behavioral and physiological functions, including memory and cognitive ability [1,2], hormone secretion [3], energy metabolism [4] and immune function [5]. Sleep deficiency has also been found to increase the risk of cardiac disease [6], diabetes [7], obesity [8], hypertension [9], cardiovascular disease [10], colitis [11], pregnancy complications [12], and neurobehavioral and cognitive impairments [13].

Ulcerations of the oral mucosa exist widely and impact patients' quality of life severely. Oral ulcers are associated with conditions such as local trauma, recurrent aphthous stomatitis (RAS), bacterial and viral infections, allergic reactions, adverse drug reactions or systemic disease [14,15]. Several studies suggested that sleep disturbances could influence oral health [16–19]. For instance, some clinical reports suggest that sleep deprivation increased risk of gingivitis [17] and periodontitis [20]. Two major reasons are proposed to account for sleep deprivation-induced oral health problems. First, subjects who are having disturbed sleep might suffer more stress, depression, anxiety and fatigue, which may increase the risk of oral disease. Second, these increased risks seem to be directly related with the neuro-immuno-endocrine system and oxidative stress alterations that were induced by sleep deprivation. Thus, we suspected that sleep deprivation might be a potential risk factor for oral health. However, the association between sleep deprivation and oral ulcer has not been well studied.

The purpose of this study is to investigate whether sleep deprivation may impair the healing process after phenol-induced oral ulcer in rats.

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2. Materials and methods

2.1. Animals

Male Sprague-Dawley rats (Eight-week-old) were purchased from Guangdong Medical Laboratory Animal Center. Animals were housed in groups of five per cage and maintained on a constant 12-h light/dark cycle with free access to food and water. Before the experiments began, the animals were habituated to maintenance conditions for one week to avoid environmental stress. All animal experiments were approved by the Animal Care and Use Committee of School of Life Sciences, Sun Yat-sen University, PR China. Adequate measures were taken to minimize pain of experimental animals.

2.2. Experimental design

Rats were randomly divided into two main groups: normal control group (n = 30), oral ulcer group (OU group, n = 50). On the 1st day, the buccal mucosa of both sides of rats in OU group was injured by 95% phenol solution after the animals were anesthetized with isoflurane. Almost uniformly round ulcers were created in the oral mucosal region on day 3. After that, the OU group was randomly split into two subgroups: oral ulcer only (OUO group, n = 20), and oral ulcer + sleep deprivation (OUS group, n = 20). The OUS group rats were subjected to sleep deprivation for 72 h from day 3 to day 6. After sleep deprivation, OUS group rats were moved to normal cages for 3 days to observe the recovery of oral ulcer after sleep deprivation. Buccal mucosa region was photographed daily after phenol injured. Body weight, food intake and body temperature measurements were performed daily at 9:00 am. The rats were sacrificed at different time points as follows: (1) Ten rats from normal control group and OU group were selected randomly and anesthetized with 10% chloral hydrate (3 mL/kg, i.p.) on day 3. (2) Ten rats of each group were performed at the same operation on days 6 and 9, respectively. Then, blood was collected from all anesthetized rats via the abdominal aortic. Serum was obtained by centrifuging the blood at 5000 rpm for 20 min at 4 °C, followed by storage at −80 °C. The buccal mucosa of both sides and whole brain tissues were obtained and frozen immediately at −80 °C. The details of experimental protocol are depicted schematically in Fig. 1.

2.3. Oral ulcer model

Oral ulceration of the rats in experimental groups was induced by the method described by Yu et al. [21] with little modification. After animals were rapidly anesthetized with isoflurane (Hebei Yipin Pharmaceutical Co., Ltd., Shijiazhuang, Hebei, China), a round plastic tube, 4 mm in diameter, was stuffed with 25 mg cotton pellets and soaked in 65 μL of 95% aqueous phenol solutions (Guangzhou Chemical Reagent Factory, Guangzhou, Guangdong, China) and pressed to the cheek mucosa of both sides for 40 s respectively. The phenol treatment induced obvious ulcer in the oral mucosal region on day 3.

2.4. Procedure for producing sleep deprivation

On the 3rd day, the OUS group rats were subjected to sleep deprivation for 72 h using a modified multiple platform technique [22]. Sleep consists of two main stages: non-rapid eye movement (non-REM) and rapid eye movement (REM) or paradoxical sleep. This method abolished REM sleep during sleep deprivation, and also resulted in 31% loss of non-REM sleep [22]. The rats were maintained in groups of ten in two separate water tanks (110 cm × 70 cm × 30 cm) contained 15 platforms (6.3 cm in diameter) rising 1 cm above the water surface. At the onset of each REM sleep episode, animals were awakened as they fell into water with the loss of postural muscle tone. All rats were habituated to sleep deprivation environment 1 h per day for one week before the start of the sleep deprivation. Food and water were available to animals by placing chow pellets and water bottles on a grid located on top of the tank. The water in the tanks was changed with clean water daily. The normal control group and OUO group were housed in standard home cages and showed normal sleep patterns. After the end of sleep deprivation, animals in the OUS group were returned to their home cages and all rats were observed for 3 days.

2.5. Measurement of ulcer area

Measurement of ulcer area was started on day 3 after ulceration. Animals were rapidly anesthetized with isoflurane, and images of oral ulcers were acquired with digital cameras. Each area was measured using Image-Pro Plus software 6.0 (Media Cybernetics Inc., Rockville, MD, USA). The degree of healing was expressed as ulcer healing rate = $(A_3 - A_t)/A_3 \times 100\%$, where A_3 and A_t represent the initial ulcer area and the ulcer area at the specific time of observation, respectively.

2.6. Measurements of food intake, body weight and body temperature

Daily food intake was measured as the difference in the mass of total food between each 24-h period. The rat's temperatures were measured using infrared thermometer with the laser sight aimed at the medial aspect of the eyes from a distance of approximately 1 cm. Body weight was measured daily using a calibrated electronic scale.

2.7. Measurements of neurotransmitters and corticosterone (CORT) in serum

Measurements of γ-aminobutyric acid (GABA), 5-

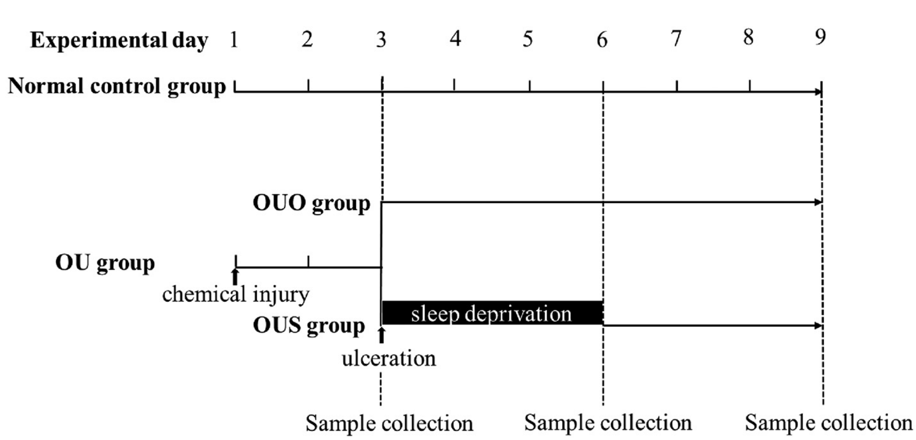

Fig. 1. Schematic representation of the experimental design. Time-line illustrations of the sequence of events throughout the experiments.

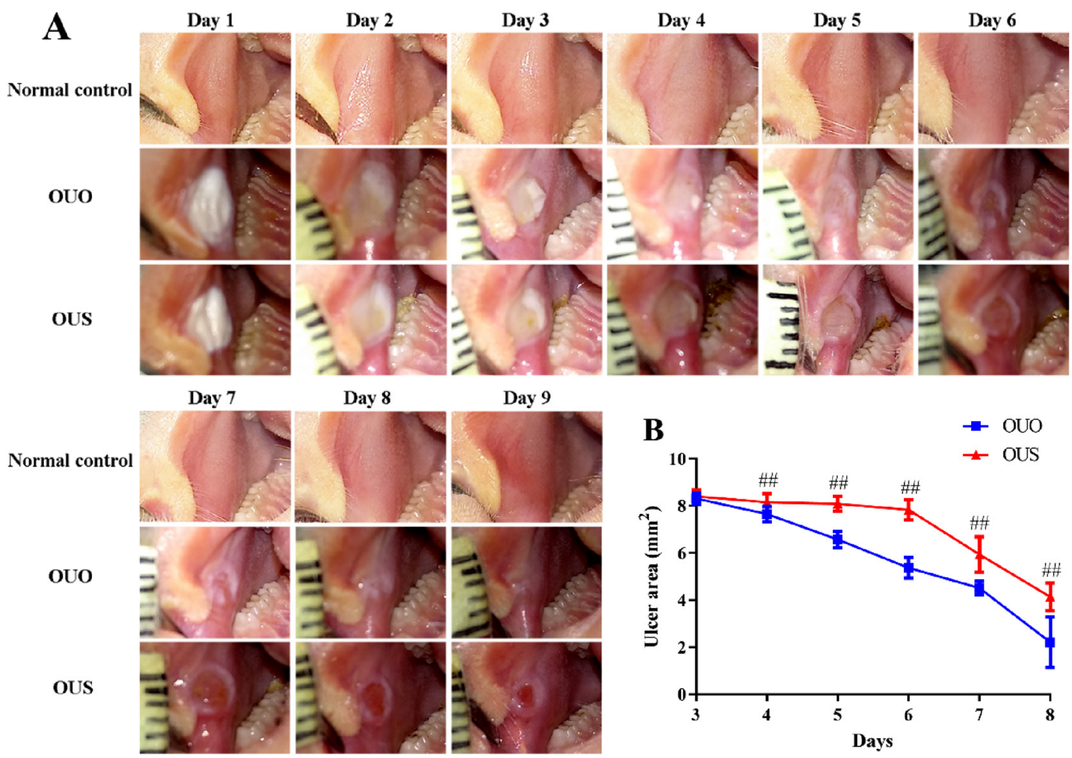

Fig. 2. Images of oral ulcers (A). Solid arrows represent ulcer regions. Oral ulcer area changes during the experiment (B). Data represent means ± SD; $^{\#\#}P < 0.01$ compared with OU group in the same day.

hydroxytryptamine (5-HT), and CORT in serum were performed using ultra-high-performance liquid chromatography-tandem mass spectrometry (LC-MS/MS) described by Chen et al. [23]. Serum samples (90 μL) were pipetted into a 1.5 mL Eppendorf tube. Then, the serum was added to 300 μL of acetonitrile (Honeywell Burdick & Jackson, Morristown, USA) with 0.1% formic acid (Sigma-Aldrich, Co., St. Louis, USA), isoprenaline hydrochloride (internal standard, IS, final concentration of 500 ng/mL, Sigma-Aldrich, Co., St. Louis, USA), and Dexamethasone (internal standard, IS, final concentration of 500 ng/mL, National Institutes for Food and Drug Control, Beijing, China) to precipitate the proteins. After vortex mixing for 5 min, the mixture was centrifuged at 13,000 rpm for 20 min at 4 °C. Ten microliters of supernatant was injected into the LC-MS/MS for analysis.

2.8. Measurements of neurotransmitters in brain tissue

Levels of GABA and 5-HT in whole brain tissue were determined using a modified version of the method described by Wojnicz et al. [24]. The brain samples were homogenized in ice-cold 1.89% formic acid in water at a concentration of 10 mL/g tissue and centrifuged at 14,000 rpm for 40 min at 4 °C. Acetonitrile with 1% formic acid and isoprenaline hydrochloride (IS, final concentration of 500 ng/mL) were added to the supernatant for protein precipitation in a 9:1 proportion (v/v) and centrifuged at 14,000 rpm for 10 min at 4 °C. Ten microliters of supernatant was injected into the LC-MS/MS for analysis.

2.9. Measurements of immunoglobulin (Ig)M, IgG and component 3 (C3) in serum

The serum IgM, IgG and C3 levels were determined by immunonephelometric assay with commercial kits (Shanghai Kehua Bio-Engineering Co., Ltd., Shanghai, China). The operations were strictly performed according to the instructions. The contents of IgM, IgG, and C3 in the sample were calculated by measuring turbidity of the reaction liquid and comparing with standard samples.

2.10. Enzyme-linked immunosorbent assay (ELISA) in serum

The concentrations of tumor necrosis factor-alpha (TNF)-α, interleukin (IL)-1β, IL-6, IL-8, monocyte chemoattractant protein-1 (MCP-1), corticotrophin (ACTH), and 8-hydroxy-deoxyguanosine (8-OHdG) in serum were detected using commercial ELISA kits (all from Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China) according to the manufacturer's instructions.

2.11. Measurements of MDA, SOD, TNF-α and IL-1β in oral mucosa tissue

The levels of malonaldehyde (MDA), superoxide dismutase (SOD), TNF-α, and IL-1β in oral mucosa tissue were detected using commercial kits (all from Nanjing Jiancheng Bioengineering Institute, Nanjing, Jiangsu, China). Tissue samples were homogenized in ice-cold normal saline in a ratio of 1/9 and then centrifuged at 5000 rpm for 20 min at 4 °C.

2.12. Histopathology

The mucosal fragments containing ulcers were collected and fixed in 4% paraformaldehyde (BioSharp, Hefei, Anhui, China), and then embedded in paraffin (Guangdong Dachuan special wax Co. Ltd., Maoming, Guangdong, China). Sections were cut in 4 μm in thickness for hematoxylin and eosin (H&E, all from Aladdin, Shanghai, China) staining. The sections were analyzed using an optical microscope (Olympus BX43; Olympus Co, Tokyo, Japan). The microscopic fields (850 μm × 650 μm) from the central region of oral ulcer in each section were chosen for histological analyses. The histological characteristics of the ulcers and the corresponding cicatrization phases were described.

2.13. Statistical analysis

Food intake data are presented as mean values, which is total food intake divided by the number of rats. The other data represent the mean

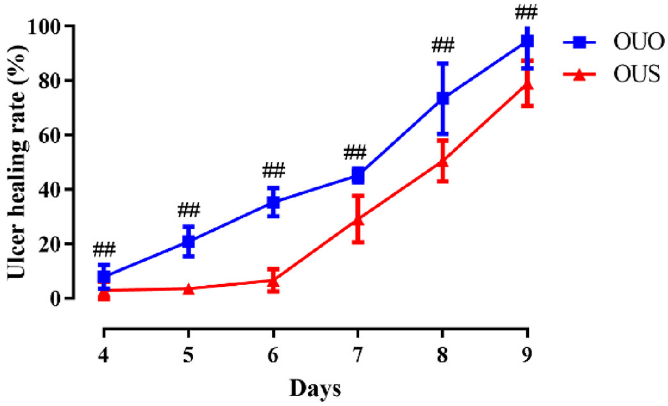

Fig. 3. Effects of sleep deprivation on ulcer healing rate. Ulcer healing rate = [(A_3 − A_t) / A_3] × 100%, where A_3 represents the initial ulcer area on day 3, and A_t represents the ulcer area on days 4–9. Data represent means ± SD; $^{\#\#}P < 0.01$ compared with OU group in the same day.

values ± standard deviation (SD) for each group. Statistical analysis was performed using the *t*-tests between two groups or one-way ANOVA followed by the Tukey test when more than two groups were compared. Results were considered significant at $P < 0.05$. The graphics presented and statistical analyses were performed using GraphPad Prism 6 program (Version 6.01).

3. Results

3.1. Effects of sleep deprivation on oral ulcer area

The images of buccal mucosa region are presented in Fig. 2A. A significant delay existed in the oral ulcer healing in the OUS group rats compared with that of the OU group rats (Fig. 2B). On the 3rd day, the baseline ulcer areas of the OU group and OUS group were (8.31 ± 0.25) mm^2 and (8.40 ± 0.28) mm^2, respectively, with no statistical difference. Sleep deprivation resulted in a significant retard of ulcer healing because ulcer areas of the OUS group during/after sleep deprivation (days 4–8) were significantly larger compared with the OUO group. As shown in Fig. 3, ulcer healing rates on days 4–9 in OUS group are higher than that in OUO group.

3.2. Effects of sleep deprivation on food intake, body weight and body temperature

As shown in Fig. 4A, chemical injury with phenol caused a decrease in food intake and sleep deprivation caused a further decrease during days 4–7. No statistical difference of body weight averages between OU group and normal control group was observed throughout the experiment. Sleep deprivation episodes sustained body weight loss and body weight of OUS group was much lower compared with other groups (Fig. 4B). As shown in Fig. 4C, body temperature had no significant changes between normal control group and OU group during the experiment. However, sleep deprivation increased the body temperature in the OUS group. Rats' body temperature was significantly higher in the OUS group than that in the other groups during days 5–7.

3.3. Effects of sleep deprivation on neurotransmitters in serum and brain tissue

The effects of sleep deprivation on neurotransmitters in serum and brain tissue are shown in Fig. 5. On the 3rd day, there was no difference in the neurotransmitters levels between groups. On the 6th day, GABA and 5-HT levels in serum and brain tissue were significantly higher in OUS group than those in the other groups. On the 9th day, the 5-HT level of brain tissue in OUS group was still higher than in normal control group; the GABA level of brain tissue showed no significant differences among the three groups. Both neurotransmitters in serum had no significant differences among the three groups on day 9.

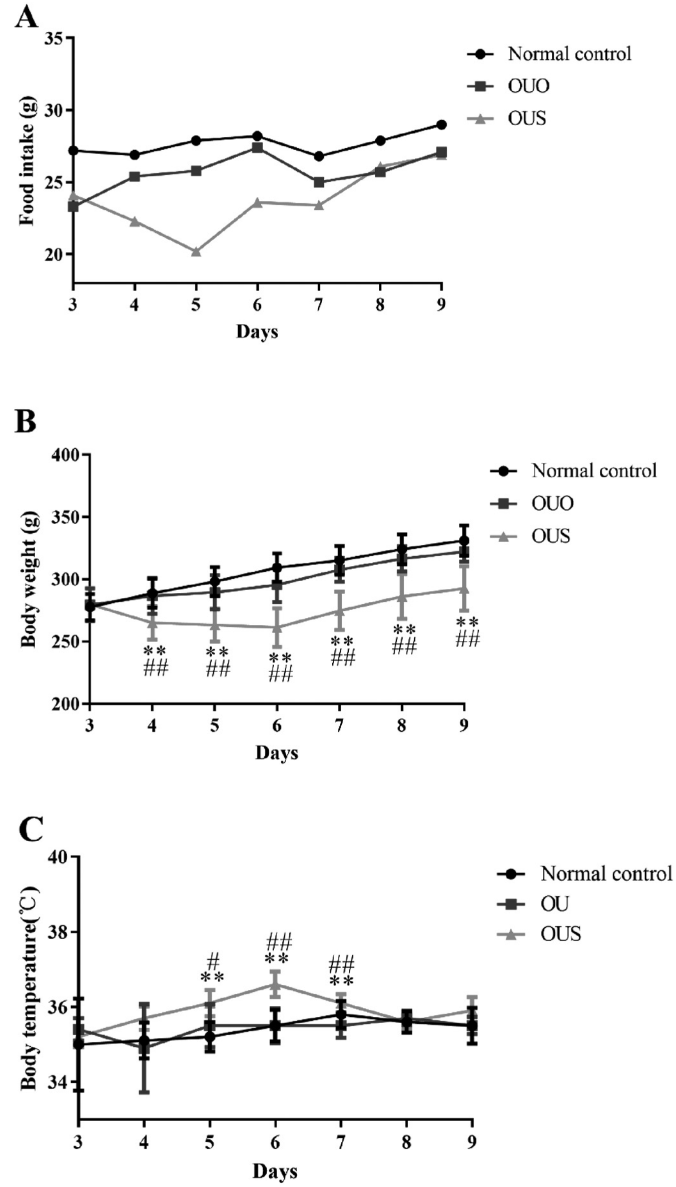

Fig. 4. Effects of sleep deprivation on food intake (A), body temperature (B), and body weight (C). Food intake data are presented as mean values, the other data are expressed as mean ± SD. $^{**}P < 0.01$ compared with normal control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with OU group.

3.4. Effects of sleep deprivation on concentrations of ACTH and CORT in serum

The effects of sleep deprivation on concentrations of ACTH and CORT in serum are shown in Fig. 6. On the 3rd day, the CORT level was significantly decreased in OU group compared with in normal control group, and the ACTH level showed no significant difference between groups. On the 6th day, serum ACTH and CORT levels in OUS group were significantly higher than that in the other groups. On the 9th day, no significant differences were observed among the three groups.

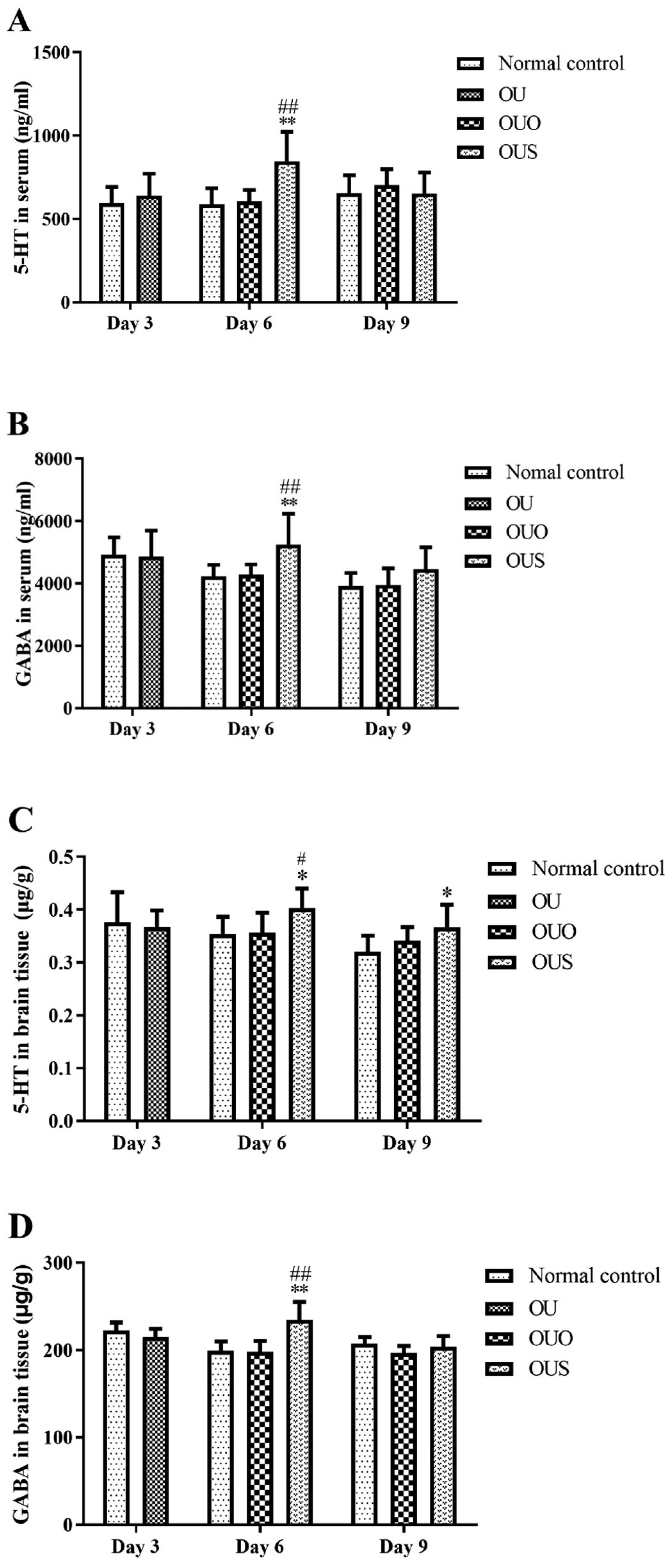

Fig. 5. The 5-HT and GABA levels in serum (A and B) and brain tissue (C and D). Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with normal control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with OU group.

3.5. Effects of sleep deprivation on concentrations of IgM, IgG, and C3 in serum

The effects of sleep deprivation on concentrations of IgM, IgG, and C3 in serum are shown in Fig. 7. On the 3rd day, in response to phenol-induced oral ulcer, C3 level in serum in the OU group was significantly increased compared with normal control group. On the 6th day, after sleep deprivation, serum IgM level in the OUS group was significantly higher compared with the normal control group. On the 9th day, serum IgM level in the OUS group was significantly higher than that in the other groups. However, IgG showed no significant differences among the three groups throughout the experiment.

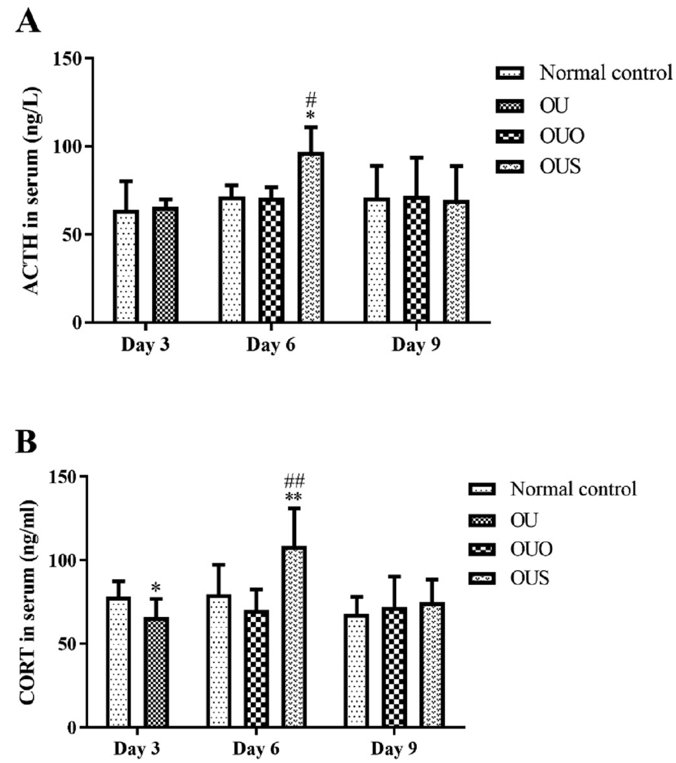

Fig. 6. The ACTH (A) and CORT (B) in serum. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the normal control group, $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with OU group.

3.6. Effects of sleep deprivation on concentrations of cytokines in serum

The effects of phenol-induced oral ulcer and sleep deprivation on inflammatory cytokines in serum were investigated in this study (Fig. 8). On the 3rd day, serum TNF-α, IL-1β, IL-6, and IL-8 levels in OU group were significantly increased compared with normal control group; however, MCP-1 level in serum showed no significant differences between groups. On the 6th day, after sleep deprivation, all serum cytokines levels were significantly higher in OUS group compared with the normal control group. Furthermore, serum IL-1β, IL-6, and MCP-1 levels in OUS group were significantly higher than those in the OU group. All cytokines showed no significant differences between OU group and normal control group. On the 9th day, IL-6 and IL-8 levels were still higher in OUS group than in normal control group and had no significant differences compared with OUO group. However, TNF-α, IL-1β, and MCP-1 showed no significant differences among the three groups on day 9.

3.7. Effects of sleep deprivation on TNF-α and IL-1β levels in buccal mucosa tissue

The effects of sleep deprivation on TNF-α and IL-1β in buccal mucosa tissue are shown in Fig. 9. On the 3rd day, buccal mucosa tissue TNF-α and IL-1β levels in OU group were significantly increased compared with normal control group. On the 6th day, buccal mucosa tissue TNF-α and IL-1β levels were significantly higher in OUS group

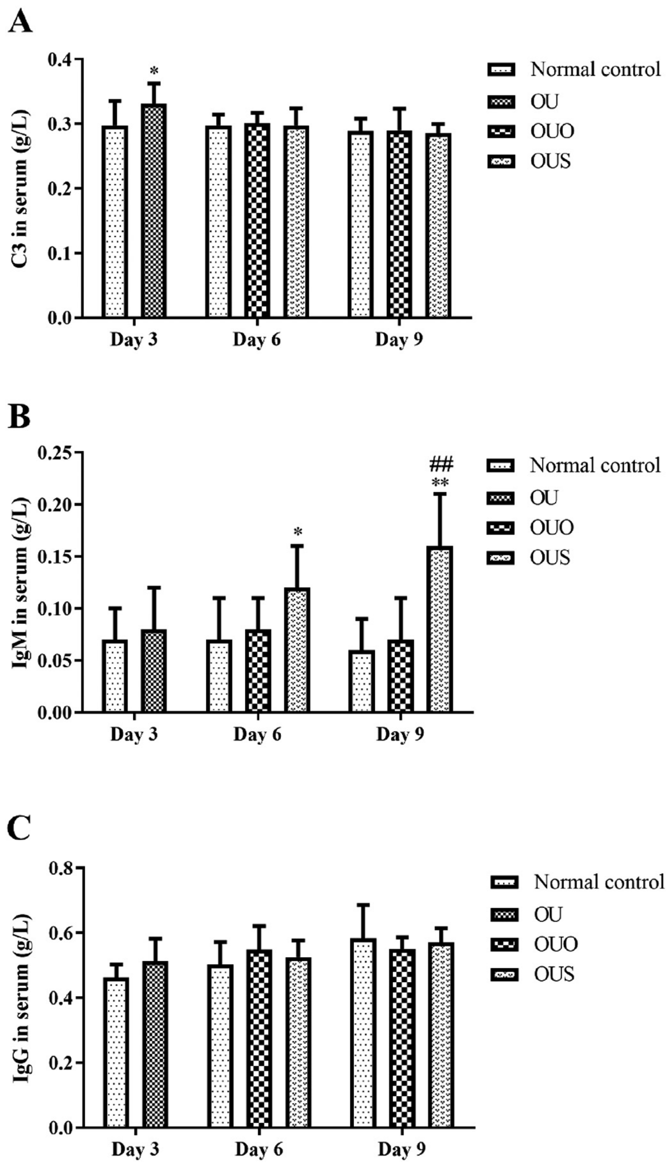

Fig. 7. The C3 (A), IgM (B), and IgG (C) in serum. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with the normal control group; $^{\#\#}P < 0.01$ compared with OU group.

compared with normal control group. Meanwhile, buccal mucosa tissue IL-1β level in OUS group was also significantly higher than that in OUO group on day 6. Both cytokines showed no significant differences between OUO group and normal control group on day 6. On the 9th day, both cytokines in buccal mucosa tissue showed no significant differences among the three groups.

3.8. Effects of sleep deprivation on MDA and SOD in buccal mucosa tissue and 8-OHdG in serum

In this study, we investigated the effects of sleep deprivation on markers of oxidative stress (Fig. 10). On the 3rd day, phenol treatment produced a significant decrease of SOD activity and increased MDA level in oral mucosa tissue in the OU group. On the 6th day, the SOD activity and MDA level in OUO group had no significant difference between normal control group and OUO group. However, after sleep deprivation, SOD activity of OUS group was significantly lower than that in normal control group; MDA level was significantly higher in OUS group than that in normal control group. On the 9th day, SOD activity and MDA level had no significant differences among the three groups. On the 3rd day, 8-OHdG levels in serum showed no significant difference between normal control group and OU group, indicating that chemical injury with phenol does not influence the 8-OHdG level in serum. On the 6th day, serum 8-OHdG level in OUS group was increased significantly compared with other groups. On the 9th day, serum 8-OHdG in serum showed no significant differences among the three groups.

3.9. Histopathology

Histopathology results are shown in Fig. 11. The histology of the healthy buccal mucosa was fully covered by stratified epithelium that was well keratinized. However, on the 3rd day, the histology of the ulcerative buccal mucosa indicated that the lamina propria was exposed due to the destruction of the mucosal surface, and it showed the loss of surface epithelium continuity (ulcerated), where homogeneous connective tissue was infiltrated with massive inflammatory cells. On the 6th day, Sleep deprivation increased inflammatory cell infiltration in OUS group, and samples from OUO group revealed similar changes to OUS group but with less inflammatory reaction. On the 9th day, histology showed that the lesions of the two groups were healed.

4. Discussion

Although, the etiology of oral ulcerations is still unknown, it has been reported that both environmental and genetic factors are responsible. It has been related with a large number of reasons involving mechanical injuries, systemic diseases, genetic predisposition, immunologic factors, hormonal level fluctuations, microelement deficiencies, nutritional factors, allergic factors, microbial infection, and psychological stress [25,26]. The present work investigated the effects of sleep deprivation on oral ulcer. This report aims to demonstrate that sleep deprivation worsened the oral ulcer and delayed oral ulcer healing.

Many studies have confirmed the crucial role of immunologic disturbances in the etiopathogenesis of RAS [27]. Previous studies indicated that sleep deficiency affects immunologic response and inflammatory mediators [28,29]. It is well known that the nervous, endocrine, and immune systems are anatomically and functionally interconnected. In addition, the hypothalamic-pituitary-adrenocortical (HPA) axis was one of the main components of this network [30]. Therefore, we studied the effects of sleep deprivation on immune inflammation and neuroendocrine systems in oral ulcer model rats.

In the present study, sleep deprivation worsened phenol-induced inflammation as evidenced by marked increase in tissue TNF-α and IL-1β levels. Meanwhile, sleep deprivation also increases serum TNF-α, IL-1β, IL-6, IL-8, and MCP-1 levels. Immune cytokines are a large and diverse group of pleiotropic and redundant polypeptides. They are rapidly induced in response to tissue injury, infection or inflammation. It has been reported that excessive production of TNF-α, IL-1β, and IL-6 were associated with an increased risk of RAS development [31–33]. IL-1 and TNF-α have been shown to induce lowering of interstitial fluid pressure, thereby facilitating edema in the skin and oral mucosa [34,35]. Hence, the involvement of cytokines in inflammation may worsen oral ulcer disease progression.

Moreover, we have also proposed a key involvement of the oxidative stress in oral ulcer healing. Our results show that sleep deprivation delayed MDA levels and SOD activity in oral mucosa tissue return to baseline levels. 8-OHdG levels in serum were also significantly increased after sleep deprivation. 8-OHdG is a biomarker substance that allows sensitive and proportional detection of oxidative DNA damage. It has been reported that the DNA damage initiates the secretion of inflammatory cytokines such as IL-6, which would promote the activation of oxidative and inflammatory mechanisms [36]. Furthermore, previous findings established that sleep deprivation results in

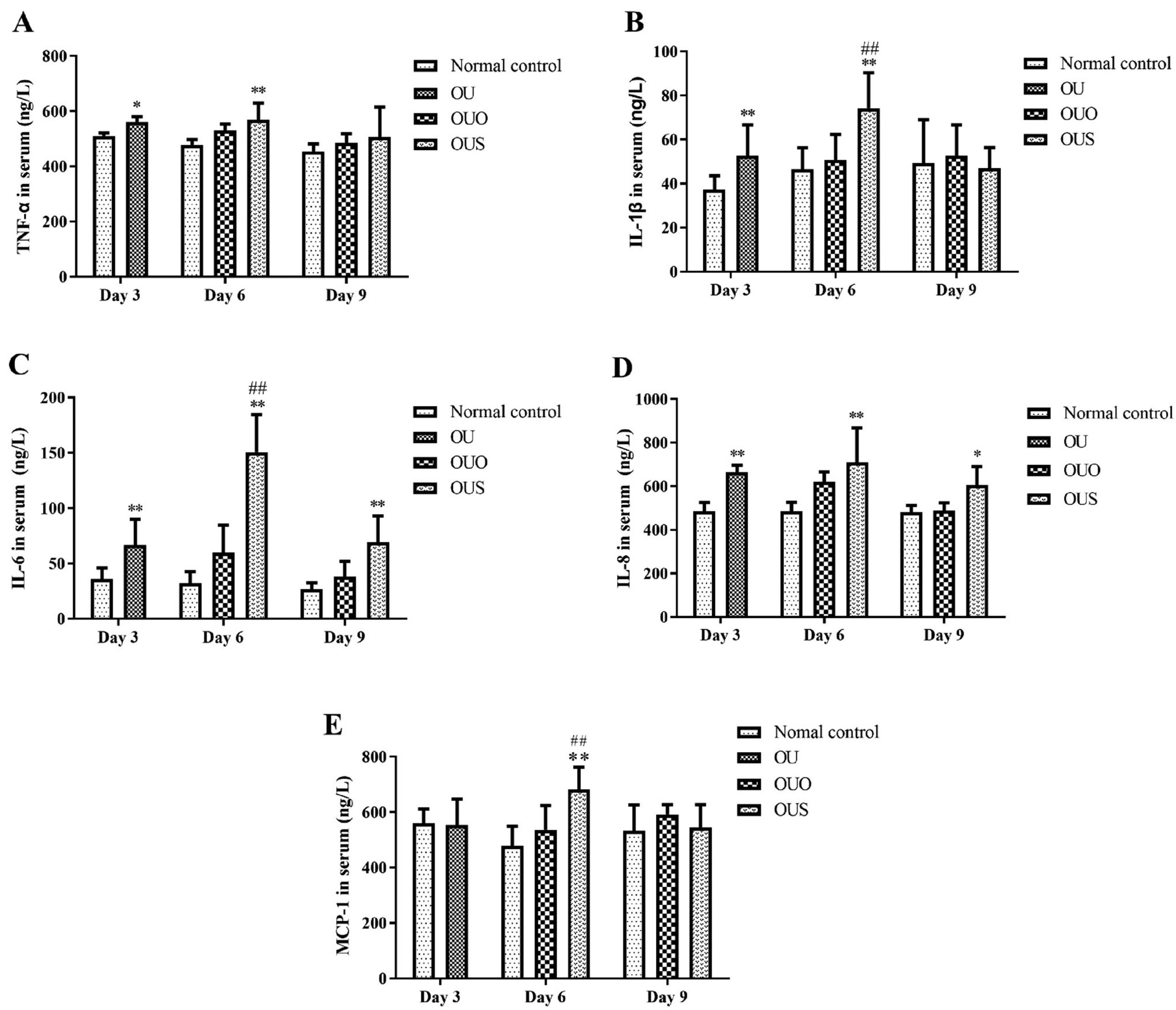

Fig. 8. The concentrations of TNF-α (A), IL-1β (B), IL-6 (C), IL-8 (D), and MCP-1 (E) in serum. Data are expressed as mean ± SD. $^{**}P < 0.01$ compared with normal control group; $^{\#\#}P < 0.01$ compared with OU group.

uncompensated oxidative stress and causes cell damage [37]. Therefore, sleep deprivation results in oxidative stress and may worsen oral ulcer.

In this study, GABA and 5-HT levels in serum and brain tissue increased significantly after sleep deprivation. GABA as a principal inhibitory neurotransmitter in the central nervous system, is closely bound to immune processes and signals [38,39]. Previous studies reported that IL-1β [40] and IL-6 [41] stimulates GABA release. Additionally, evidence suggests involvement of GABAergic modulation in sleep deprivation-induced oxidative damage [42]. It is therefore feasible that sleep deprivation-induced increased pro-inflammatory cytokines levels and oxidative stress may contribute to increasing GABA release. However, Minano et al. [43] found that oral and intraperitoneal administration of GABA exhibited antiulcer activity in pyloric ligation-induced gastric ulcers rats. It is noteworthy that GABA has been shown to promote wound healing in model rats through topical application of GABA on wounds [44]. Recently, Xie et al. [45] reported that GABA showed protective effects on ethanol-induced gastric mucosal injury in rats which were given different dose (10, 20 and 40 mg/kg/day) of GABA by oral administration. The GABA concentrations in rats treated with exogenous GABA (orally or intraperitoneally) may be much higher than that in sleep deprived rats. Thus, we consider that GABA in sleep deprived rats may not be enough to inhibit ulcers in this study. On the other hand, sleep deprivation increased pro-inflammatory cytokine levels and induced oxidative stress in this study. Therefore, although GABA levels were increased, sleep deprivation could still worsen oral ulcer by increasing pro-inflammatory cytokines and oxidative stress. 5-HT, which has long been considered to have an important role in the control of pain, is released from platelets, mast cells and endothelial cells into an injured site during inflammation in the central nervous system and in the periphery [46]. Furthermore, 5-HT is one of important pronociceptive mediators which produce inflammation and hyperalgesia or allodynia [47,48]. One report suggested that 5-HT presented in bronchial epithelium of smokers may be involved in cigarette smoke-induced oxidative stress and inflammation by activating p38 MAPK and ERK pathway [49]. Therefore, this result suggests that endogenous 5-HT may be involved in the aggravating action of oral ulcers by producing inflammation and hyperalgesia.

Our data demonstrate that body temperature increased significantly from baseline during sleep deprivation, and the alterations were reversed by sleep recovery. Hyperthermia is the hallmark of inflammation, and previous studies suggested that cytokines (such as TNF-α, IL-1β and IL-6) played important roles as being responsible for temperature increase [50–52]. Accordingly, the effect of hyperthermia during

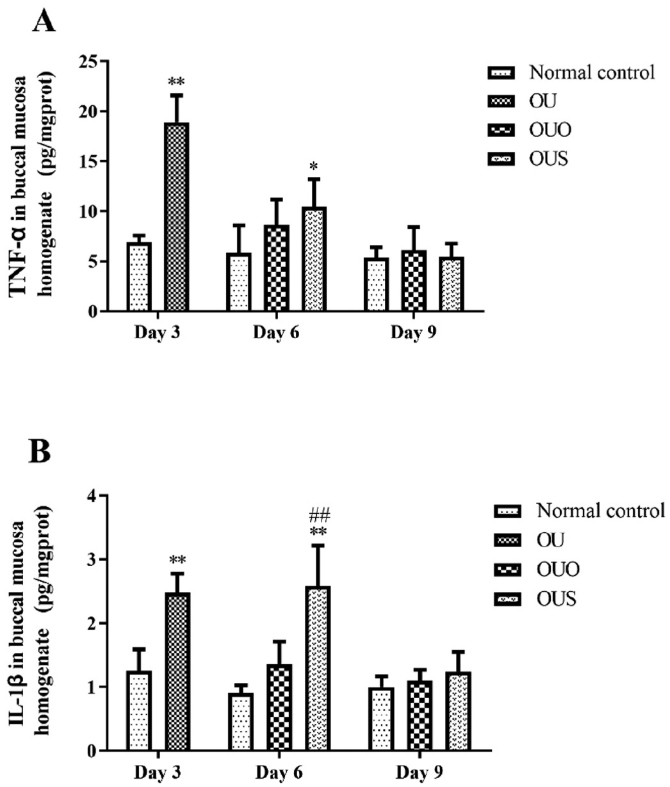

Fig. 9. The TNF-α (A) and IL-1β (B) levels in buccal mucosa tissue. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with normal control group; $^{\#\#}P < 0.01$ compared with OU group.

sleep deprivation might be mediated by the sleep deprivation-induced inflammatory response.

Food intake in OUO group was decreased compared with normal control group, and we speculate that the result might be related to the pain caused by ulcerative oral lesions. However, the present finding indicates that sleep deprivation further decreased the total food intake inconsistent with other reports [53–55]. The possible reason is that sleep deprivation may produce hyperalgesia [56] in oral ulcer rats compared to other studies performed sleep deprivation in normal rats. On the one hand, 5-HT could trigger sensitization of primary nociceptive afferent fibers as well as nociceptive neurons from which these fibers originate in dorsal root ganglia, thereby contributing to peripheral sensitization and hyperalgesia [57]. On the other hand, there is growing evidence that cytokines are involved in the generation of pain and hyperalgesia [58]. Therefore, sleep deprivation may aggravate oral ulcer-induced mechanical pain hypersensitivity through up-regulation of 5-HT and cytokines levels which lead to anepithymia in oral ulcer rats.

Body weight declined progressively during the sleep deprivation period. This trend was the same as that observed in previous studies [59,60]. It has been reported that sleep deprivation could produce increased energy expenditure and loss of fat content [59]. Meanwhile, food intake was decreased in OUS group compared with OUO group.

According to our data, sleep-deprived rats exhibited increased levels of serum CORT and ACTH, showing adequate HPA stress responsiveness. Our results are consistent with other reports [28,59] demonstrating that the sleep deprivation can activate HPA axis. An effective HPA axis activity and adequate sleep are crucial for the control of excessive inflammatory and immune conditions [61]. The deleterious effects of sleep deprivation on oral ulcer probably involve dysregulation of the HPA axis.

In addition, IgM level in serum has been found to be increased in OUS group. Our results suggest immune IgM was secreted following

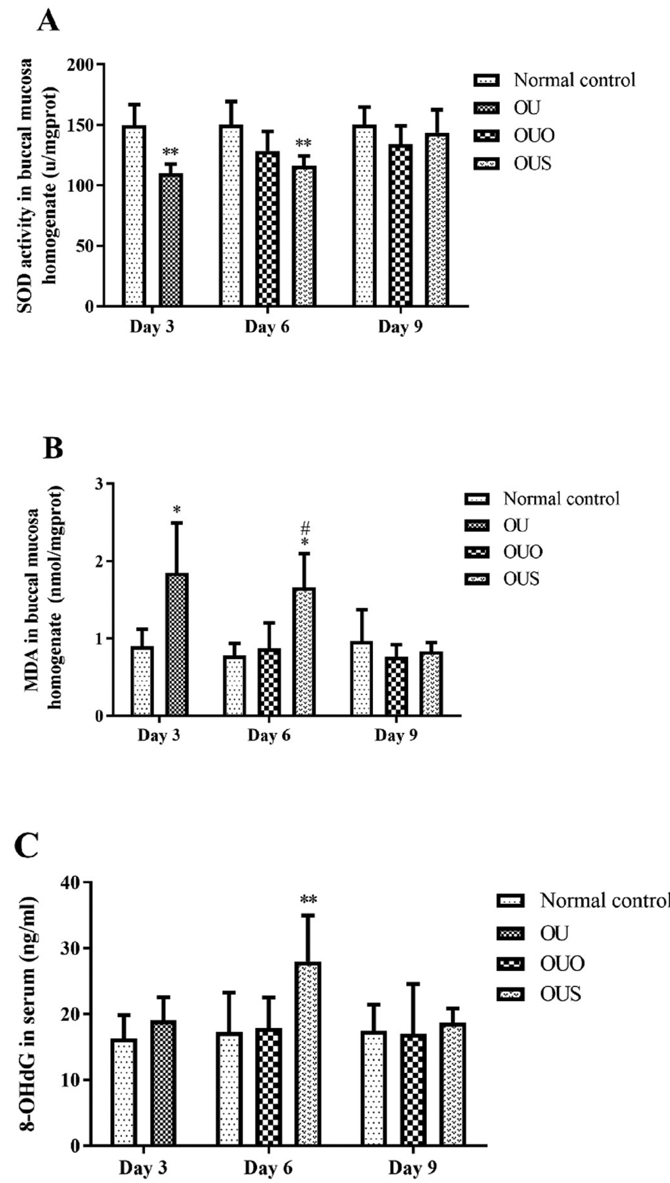

Fig. 10. The SOD activities (A) and MDA (B) levels in buccal mucosa tissue and 8-OHdG (C) levels in serum. Data are expressed as mean ± SD. $^{*}P < 0.05$, $^{**}P < 0.01$ compared with normal control group; $^{\#}P < 0.05$, $^{\#\#}P < 0.01$ compared with the OU group.

exposure to sleep deprivation and further increased after sleep deprivation. The results are in agreement with previous study demonstrating an increase in serum IgM levels in acute sleep deprivation humans [62]. In this study, sleep deprivation does not influence the serum IgG and C3 levels in rats. Our data suggest that sleep deprivation leads to alterations of immune system evidenced by excessive production of pro-inflammatory cytokines and IgM.

5. Conclusion

In present study, our findings suggest that sleep deprivation can worsen the oral ulcers and delay oral ulcer healing. To our knowledge, this is the first study reporting the effects of sleep deprivation on the phenol-induced oral ulcer model in rats. The present study suggests that neuro-immuno-endocrine system may involve in worsening oral ulcer. In addition, another possible reason for sleep deprivation-induced deterioration of oral ulcers is the promotion of oxidative stress of the host. However, the mechanism through which sleep deprivation worsens oral

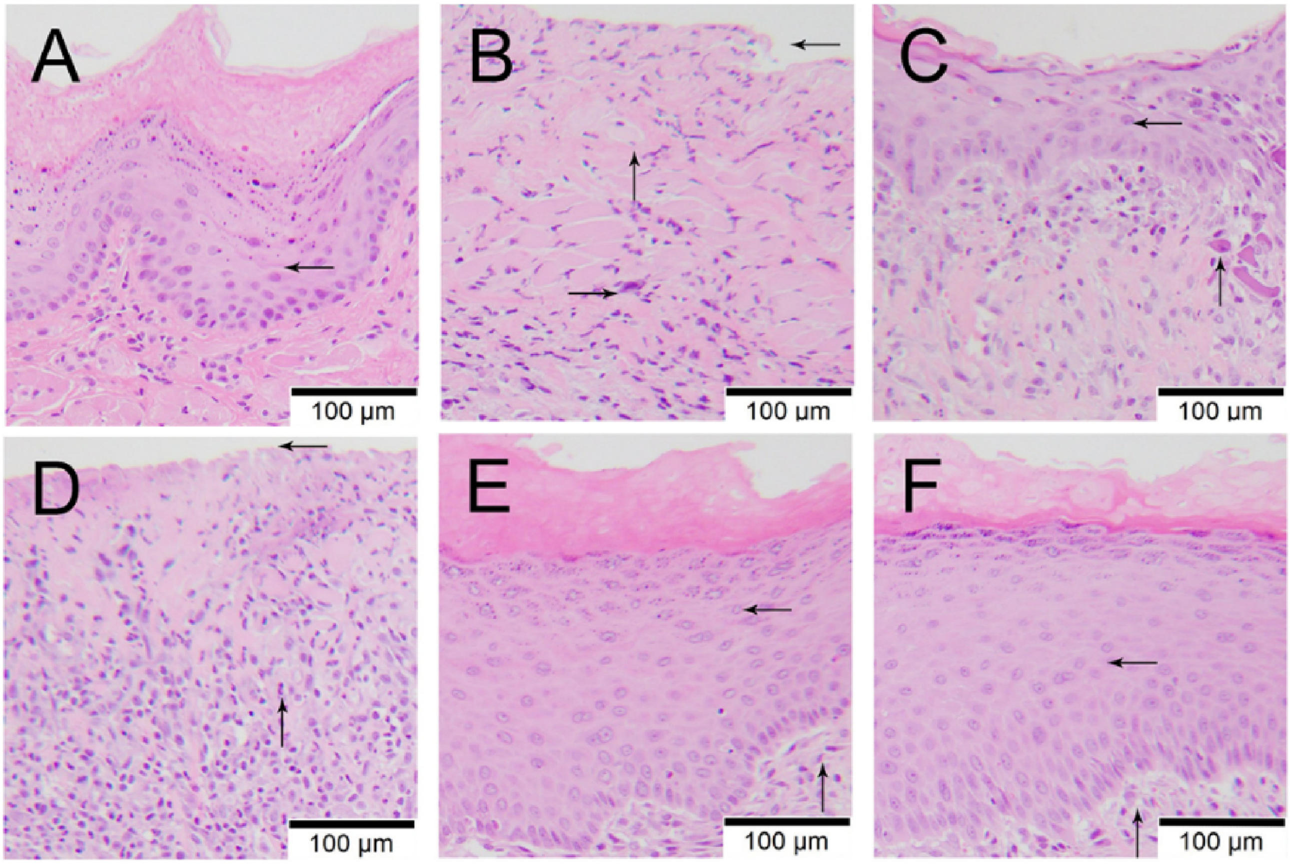

Fig. 11. Histology of the buccal mucosa. (A) Normal control group, showing normal epithelium with thin keratin layer (←), (B) OU group on the 3rd day, showed loss of surface epithelium (←), watery edema of the lamina propria (↑), fibrous hyperplasia accompanied inflammatory infiltrate (→), (C) OUO group on the 6th day, showed re-epithelialization (←) and connective tissue with inflammatory infiltrate (↑), (D) OUS group on the 6th day, showed increased inflammatory cell infiltration (↑), (E) OUO group on the 9th day, showed intact mucosa with a thin keratin layer (←) and fibrous hyperplasia (↑), (F) OUS group on the 9th day, showed intact mucosa with a thin keratin layer (←) and fibrous hyperplasia (↑).

ulcers is not known and requires further studies.

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Declaration of Competing Interest

None.

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