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Original Article

Ghrelin alleviates depression-like behaviour in rats subjected to high-fat diet and diurnal rhythm disturbance

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Abstract: Objectives: In the era of globalization, a sedentary lifestyle is highly linked with obesity and neurobehavioral complications such as depression. While depression is associated with dopamine dysfunction in the ventral tegmental area (VTA), ghrelin enhances the dopaminergic activity in the VTA. Therefore, the present study aimed to assess the effect of ghrelin on depression-like behaviour in rats subjected to a high-fat diet (HFD) and disturbed diurnal rhythm (DDR) for 45 days. Methods: The neurobehavioral deficits resulting from HFD and DDR in rats, and the behaviour modulation by intra-VTA administration of ghrelin, alone or in combination with ghrelin receptor antagonist were confirmed by evaluation of behavioural parameters in the elevated plus-maze, forced swim test, open field test, and rotarod assessment. Further, the levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and IL-6, oxidative stress marker malondialdehyde (MDA), and antioxidants enzymes like superoxide dismutase (SOD), reduced glutathione (GSH), and catalase (CAT) were measured. Results: The levels of TNF-α, IL-1β, IL-6, and MDA were increased in the brain of HFD and DDR exposed rats, while that of SOD, GSH, and CAT were reduced. Intra-VTA ghrelin administration from day 41-45 to the HFD and DDR exposed rats improved cognitive behaviour and physical activity confirming the antidepressant effect. Moreover, ghrelin restored the levels of SOD, GSH and CAT efficiently, and reduced that of MDA, TNF-α, IL-1β and IL-6, which signifies its protective effect. Conclusion: Overall, this study confirmed the ameliorative effect of ghrelin in HFD- and DDR-induced depression-like behaviour.

Keywords: High-fat diet, diurnal rhythm disturbance, ghrelin, depression, cytokines

Introduction

The rapid urbanization of the world in recent decades has significant implications for human health. In 2018, the United Nations Department of Economic and Social Affairs estimated that 55% of the global population currently lives in urban areas, and the figure is predicted to reach about 68% by 2050 [1]. Due to urbanization, the incidences of obesity, diabetes mellitus, and cardiovascular disorders have been increasing at an alarming rate, which are associated with irregular sleep habits and sedentary lifestyles. These factors also contribute substantially to the development of depression and other mental disorders. Depression is a serious mental disorder characterized by several health problems including, but not limited to anhedonia and changes in appetite [2-4]. It carries immense psychological, functional, social and other burdens, and represents the leading cause of global disability. Chronic consumption of calorie-rich food can distinctly lead to depression-like behaviour and modifications in brain reward circuity [5]. Moreover, crippled or abnormal diurnal rhythms in multiple body functions are associated with depressive disorders [6].

Ghrelin is an important gastrointestinal peptidergic hormone, which serves as an endogenous ligand for the growth hormone secretagogue receptor (GHSR) or ghrelin receptor [7]. GHSR is extensively distributed in the hypothalamus and pituitary gland. Ghrelin acts primarily at these sites to stimulate growth hormone release [8]. Interestingly, diurnal variations shown by ghrelin in the stomach and systemic circulation are reported to be influenced by feeding rhythms, which suggest a role for this peptide as a meal initiator [9]. While dopamine dysfunction in the ventral tegmental area (VTA) is associated with obesity, addiction, and depression [10, 11], ghrelin produces a direct effect on VTA neuronal activity by stimulating the dopaminergic cells [12]. The GHSR and dopaminergic D1 receptors are co-expressed in the same neurons, and ghrelin has the ability to amplify dopamine signalling by a mechanism associated with a heterodimer formation of the two receptors [13]. Recent studies reported that the ghrelinergic system is involved in the modulation of depression. Increased ghrelin levels by chronic stress might serve as a counter-regulatory mechanism to decrease anxiety- and depression-like behaviours [14]. Also, central administration of ghrelin caused alleviation of anxiety- and depression-like behaviours triggered by chronic unpredictable mild stress [15].

Since high-fat diet (HFD) feeding and disturbed diurnal rhythm (DDR) can help to mimic the possible features of depression in the rodents [16, 17], there is a need for more investigation to establish the role of ghrelin in the framework of the VTA in the underlying mechanisms responsible for the development of depression. With this background, the present study aimed to investigate the ameliorative potential of intra-VTA ghrelin in depression-like behaviour induced by concomitant exposure of rats to HFD and DDR. Moreover, the effects of ghrelin on the levels of pro-inflammatory cytokines such as tumor necrosis factor-α (TNF-α), interleukin-1β (IL-1β) and interleukin-6 (IL-6), antioxidants enzymes like superoxide dismutase (SOD), reduced glutathione (GSH) and catalase (CAT), and oxidative stress marker malondialdehyde (MDA) were measured in the brain of rats with depression-like behaviour. It may be noted that the increased levels of pro-inflammatory cytokines and oxidative stress contribute to the development of depression [18, 19].

Materials and methods

Animals

Male Sprague-Dawley rats (180-200 g) were kept at standard laboratory conditions under natural light-dark cycle, humidity (50±5%), and room temperature (25±2°C). The animals were fed with chow pellet diet (Nutrivet Life Sciences, Pune, India) with drinking water *ad libitum*. The study was approved by the Institutional Animal Ethics Committee, R. C. Patel Institute of Pharmaceutical Education and Research, Shirpur, Maharashtra, India (Approval No. IAEC/CPCSEA/RCPIPER/2017-22).

Drugs and chemicals

Ghrelin and ghrelin antagonist (H-His-D-Trp-D-Lys-Trp-D-Phe-Lys-NH2) were purchased from Peptide International (Kentucky, USA). The drug solution was dissolved in saline and kept at -20°C. Ketamine (Themis Medicare Ltd.), xylazine (Brilliant Bio. Ltd.), 5-5-dithiobis-(2-nitrobenzoic acid), and thiobarbituric acid (Loba Chemie Pvt. Ltd.) were purchased from the local market. All chemicals used for the analytical studies were bought from Sigma Aldrich (St. Louis, USA).

Experimental design

The experiment was performed to evaluate whether an acute intra-VTA administration of ghrelin modifies the performance of rats exposed to concomitant HFD and DDR in the open field test (OFT), forced swim test (FST), elevated plus maze (EPM), and rotarod test in relation to those infused with saline. The animals received ghrelin (1 μg/μl, intra-VTA) [20] or saline (control) for the last 5 days of the study (day 41-45) to evaluate its antidepressant-like action.

Stereotaxic surgery

Rats were anesthetized by intraperitoneal injection of ketamine (50 mg/kg) and xylazine (10 mg/kg) and placed in a stereotaxic apparatus (Harvard Apparatus). Stainless steel guide cannula prepared in-house [21, 22] was implanted in the VTA (-5.6 mm anterio-posterior, 2.1 mm medio-lateral, and -8.5 mm dorso-ventral at 10° angle to the vertical) [21, 23]. The cannula was fixed to the skull surface with

Table 1. Experimental groups

Group No.	Exposure/Treatment	Abbreviation
Group 1	Normal chow diet + normal diurnal rhythm	ND+NR
Group 2	Sham + disturbed diurnal rhythm	SHAM+DDR
Group 3	Normal chow diet + disturbed diurnal rhythm	ND+DDR
Group 4	High-fat diet + normal diurnal rhythm	HFD+NR
Group 5	High-fat diet + disturbed diurnal rhythm	HFD+DDR
Group 6	High-fat diet + disturbed diurnal rhythm + ghrelin	HFD+DDR+GHR
Group 7	High-fat diet + disturbed diurnal rhythm + ghrelin + ghrelin antagonist	HFD+DDR+GHR+ANTA

anchoring screws and dental acrylic cement. Each animal was then kept in a separate cage. After surgery, the rats were subjected to the dietary and environmental manipulations as described in the following section for 40 days. Thereafter, the animals were injected with ghrelin or saline (intra-VTA) using a Hamilton microliter syringe connected by PE-10 polyethylene tubing to a 30-gauge needle extending 0.75 mm beyond the guide cannula. Each infusion of 1 µl was delivered over a 1 min period. The methods of stereotaxic cannulation and central drug administration are already standardized in our earlier studies [21, 23, 24].

Diurnal rhythm disturbance (DDR) and high-fat diet (HFD) feeding

Throughout the study, the animals were placed under a 10 h light:14 h dark cycle with alternate changes in light and dark for disturbance in diurnal rhythm [25, 26]. In parallel, the rats were fed with HFD (Research Diet Inc., NJ, USA) for induction of depression [27].

Experimental groups

The animals were divided into 7 experimental groups and subjected to dietary manipulations and/or environmental changes for a period of 45 days as shown in **Table 1**. The ghrelinergic agents were injected daily for the last 5 days of the experiment in Groups 6 and 7. While treatment with ghrelin (1 µg/µl, intra-VTA) was given to Group 6, ghrelin antagonist (1 µg/µl, intra-VTA) was administered 15 min prior to ghrelin (1 µg/µl, intra-VTA) in case of Group 7.

Body weight measurement

Body weight of all groups of animals was measured on days 1, 15, 30, 40, and 45 of the study using the electronic weighing scale and reported as changes in the body weight during the experimental protocol.

Forced swim test (FST)

FST was performed according to the method described earlier [28, 29]. A glass cylindrical tank (45×28 cm) was filled with water (25± 0.1°C) to a depth of 30 cm, and the light was dimmed during the test. Two swimming sessions were conducted: an initial 15 min pre-test followed 24 h later by a 5 min test. Video recordings were scored using Any-Maze Software for time spent immobile, swimming, climbing, and latency to immobility.

Elevated plus maze (EPM)

The EPM consisted of two open arms (50 cm×10 cm) and two closed arms (50 cm×10 cm, surrounded by 40 cm wooden walls) that originated from a common central platform (10 cm×10 cm). The apparatus was raised to a height of 50 cm above the floor. The rat was placed on the central platform with the head facing towards an open arm and allowed to explore for 5 min [30]. The time spent in the open arms and closed arms were scored using Any-Maze Software.

Open-field test (OFT)

The open field was carried out using a square wooden box measuring 75×75×40 cm with red walls and a white smooth polished floor divided by black lines into 16 equal squares 4×4. Each rat was placed gently in the central area of the open field and allowed to freely explore the area for 5 min. The floor and walls were cleaned after testing each rat to eliminate possible bias because of odours left by previous rats. Any Maze Video Tracking System was fixed on the top of the box to record the move-

ment and behaviour of rats for later off-line analysis. Behavioural changes namely mobility time and immobility time were recorded [31].

Rotarod

Animals were monitored for motor coordination and balance with the help of a rotarod apparatus (7 cm in diameter and rotating at a constant speed of 20 RPM). Initially, animals were habituated to maintain posture on the rotarod by giving two training sessions of 5 min each with a gap of 10 min between the two sessions. After training, animals were allowed to move over the rotarod and their latency of falling time was recorded using a cut-off limit of 300 s [32].

Brain biochemistry

At the end of the experiment, the brain of each rat was isolated, weighed, and stored in liquid nitrogen and 10% homogenate was prepared in ice-chilled phosphate buffer (PH 7.4). The homogenate was centrifuged at 5000 RPM for 20 min at 4°C, and the supernatant was used for further analysis of thiobarbituric acid reactive substances like SOD, GSH, CAT, MDA, TNF-α, IL-1β and IL-6.

Thiobarbituric acid reactive substances

Lipid peroxidation in the brain was determined by measuring thiobarbituric acid reactive substances as MDA content according to the method described previously [33]. Briefly, 0.2 ml of tissue homogenate was mixed with 0.2 ml of 8.1% sodium dodecyl sulfate, 1.5 ml of 30% acetic acid, and 1.5 ml of 0.8% thiobarbituric acid. The reaction mixture was heated for 60 min at 95°C and then cooled on an ice bath. After cooling, 1.0 ml of distilled water and 5.0 ml of n-butanol:pyridine (15:1 v/v) were added and centrifuged at 5000 RPM for 20 min. The absorbance of the organic layer was measured at 532 nm. The levels of malondialdehyde were expressed as μg/mg of protein.

Estimation of reduced glutathione (GSH)

The GSH was estimated using a procedure given earlier [34] with brief modification as 100 μl of tissue homogenate, mixed with 100 μl of 10% trichloroacetic acid and vortexed. The content was then centrifuged at 5000 RPM for 10 min then; the reaction mixture was mixed with 3.0 ml 0.3 M phosphate buffer (PH 7.4). The absorbance was measured using a spectrophotometer at 412 nm. The levels of GSH were expressed as μg/mg of protein.

Evaluation of catalase (CAT)

Catalase activity was measured using the method described previously [35], 50 μl of tissue supernatant and mixture of 1.0 ml of 50 mM phosphate buffer (PH 7.4) and 0.1 ml of 30 mM hydrogen peroxide were added. The absorbance was read as a reduction in optical density on every 5 s for 30 s at 240 nm. The activity of catalase was expressed as U/μg of protein.

Estimation of superoxide dismutase (SOD)

The SOD activity was determined with slight alteration and according to the protocol given earlier [36]. Twenty-five microliters of tissue supernatant and a mixture of 100 μl of 500 mM Na_2CO_3, 100 μl of 1 mM EDTA, 100 μl of 240 μM NBT, 640 μl of distilled water, 10 μl of 0.3% TritonX-100 and 25 μl of 10 mM hydroxylamine was added. The readings were recorded using a spectrophotometer in kinetic mode at an interval of 1 min up to 3 min at 560 nm. The enzyme activity was expressed as U/μg of protein.

Estimation of cytokines

Standard, control, and tissue homogenate were pipetted into a microplate well pre-coated with monoclonal antibodies specific for rat TNF-α, IL-1β and IL-6. The remaining protocol was followed as given in the manufacturer's instruction manual. The intensity of the color was measured using an ELISA microplate reader (PowerWave XS). The activity of TNF-α, IL-1β and IL-6 was expressed as pg/mg of protein.

Histology

Brain tissue was fixed in buffered formalin solution and embedded in paraffin. The serial sections were cut using a microtome. Each section was stained with hematoxylin and eosin and examined under the light microscope and digital images were captured using motic image software 2.0. The pathologist performing the microscopy was unaware of the treatment and experimental groups.

Statistical analysis

The data were expressed as mean ± standard error mean (SEM). The statistical significance

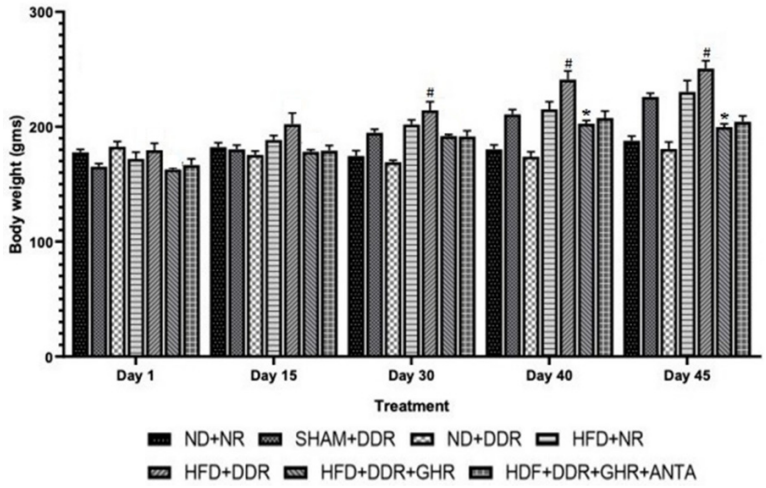

Figure 1. Effect of ghrelin on changes in body weight of rats subjected to HFD and DDR. The data were expressed as mean ± SEM, and analyzed by one-way ANOVA followed by Bonferroni's post hoc test. #P<0.001 vs. ND+NR; *P<0.001 vs. HFD+DDR. ANOVA, analysis of variance; ANTA, ghrelin antagonist; DDR, disturb diurnal rhythm; GHR, ghrelin; HFD, high-fat diet; ND, normal diet; NR, normal rhythm; SEM, standard error of the mean.

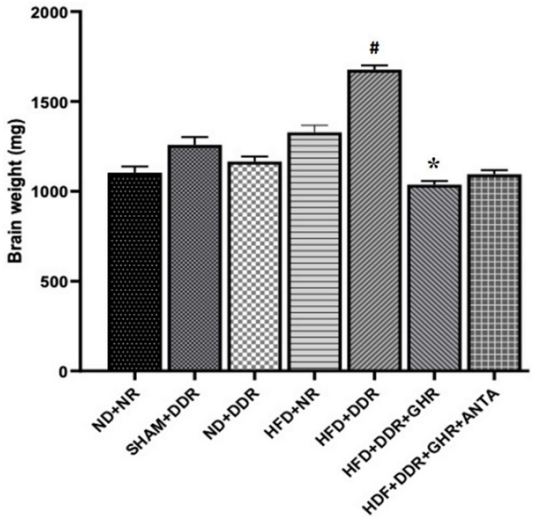

Figure 2. Effect of ghrelin on brain weight of rats subjected to HFD and DDR. The data were expressed as mean ± SEM and analyzed by one-way ANOVA followed by Bonferroni's post hoc test. #P<0.001 vs. ND+NR; *P<0.001 vs. HFD+DDR. ANOVA, analysis of variance; ANTA, ghrelin antagonist; DDR, disturb diurnal rhythm; GHR, ghrelin; HFD, high-fat diet; ND, normal diet; NR, normal rhythm; SEM, standard error of the mean.

was analyzed by one-way analysis of variance (ANOVA) followed by Bonferroni's post hoc test using a graph pad prism software, version 6.0, USA. P<0.05 was considered statistically significant.

Results

Effect of ghrelin on changes in body weight of rats subjected to HFD and DDR

The HFD+DDR group showed a significant (P<0.001) increase in weight on the 30th, 40th and 45th days when compared with the ND+NR control group. After treatment with ghrelin (HFD+DDR+GHR), a significant reduction in body weight was observed on the 40th and 45th day when compared with the HFD+DDR group (**Figure 1**).

Effect of ghrelin on brain weight of rats subjected to HFD and DDR

The brain weight was significantly increased in the HFD+DDR group (P<0.001) as compared with the normal ND+NR group. A significant decrease (P<0.001) in brain weight was noticed with ghrelin treatment (HFD+DDR+GHR) as compared to the HFD+DDR group (**Figure 2**).

Effect of ghrelin on changes in behavioral parameters of rats subjected to HFD and DDR

The FST (**Figure 3A**) and OFT (**Figure 3B**) were used to measure the immobility time in the experimental groups. The immobility time of the HFD+DDR group was significantly (P<0.001) increased when compared ND+NR group. Treatment with ghrelin (HFD+DDR+GHR) significantly reduced (P<0.001) the immobility time as compared to the HFD+DDR group. Also, the EPM test showed a significant increase (P< 0.001) in closed arm time of the HFD+DDR group when compared to the ND+NR group (**Figure 3C**). A significant decrease (P<0.001) in closed arm time was observed in the HFD+DDR+GHR group when compared with the HFD+DDR group. In the rotarod test, the latency to fall was significantly decreased (P<0.001) in the HFD+DDR group when compared with the the ND+NR group. A significant increase (P<0.001) in latency was observed in the HFD+DDR+GHR group when compared with the HFD+DDR group (**Figure 3D**).

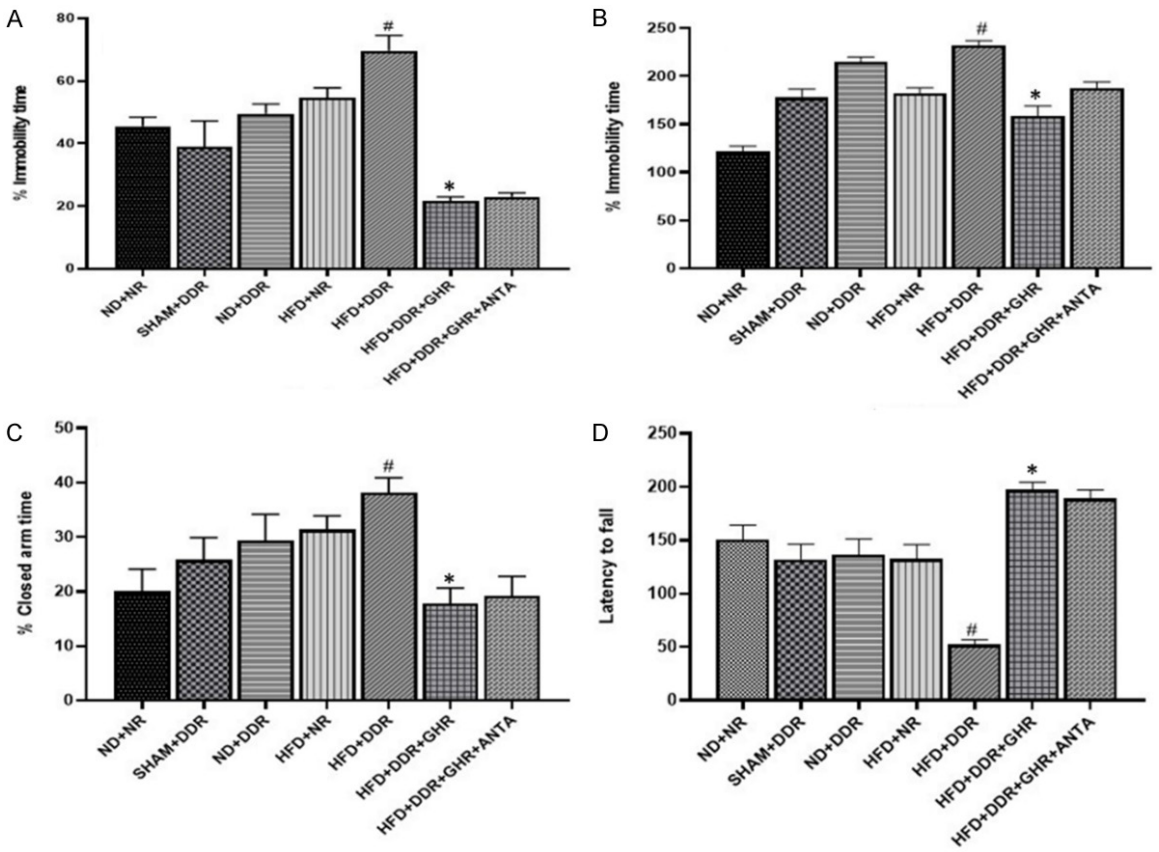

Figure 3. Effect of ghrelin on changes in behavioral parameters (A: % immobility in FST, B: % immobility in OFT, C: % closed arm time in EPM test, D: latency to fall in rotarod test) in HFD and DDR induced depression in rats. The data were expressed as mean ± SEM and analyzed by one-way ANOVA followed by Bonferroni's post hoc test. #P<0.001 vs. ND+NR; *P<0.001 vs. HFD+DDR. ANOVA, analysis of variance; ANTA, ghrelin antagonist; DDR, disturb diurnal rhythm; GHR, ghrelin; HFD, high-fat diet; ND, normal diet; NR, normal rhythm; SEM, standard error of the mean.

Effect of ghrelin on oxidative stress in the brain of rats subjected to HFD and DDR

A significant reduction (P<0.001) in SOD, GSH and CAT was observed in the HFD+DDR group as compared to the normal ND+NR group. Treatment with ghrelin (HFD+DDR+GHR) showed a significant (P<0.001) increase in the levels of these markers as compared to the HFD+DDR group. Increased lipid peroxidation was evidenced by elevated MDA levels in the HFD+DDR group as compared to the normal group. Treatment with ghrelin significantly (P<0.001) inhibited lipid peroxidation as compared to rats in the HFD+DDR group (**Table 2**).

Effect of ghrelin on cytokine levels in the brain of rats subjected to HFD and DDR

TNF-α, IL-1β and IL-6 levels were relatively high (P<0.001) in the HFD+DDR group as compared to the normal control group (ND+NR). Treatment with ghrelin (HFD+DDR+GHR) significantly lowers (P<0.001) the levels of the pro-inflammatory cytokines as compared with the HFD+DDR animals (**Table 3**).

Effect of ghrelin treatment on histopathology of the brain of rats subjected to HFD and DDR

The HFD+DDR group showed abnormal structure and architecture of the brain cells. It also showed an increase in the infiltration of the cells with edema. The rats treated with ghrelin showed normal structure and architecture as evidenced by a reduction in the spaces between neurons and infiltration of cells (**Figure 4**).

Discussion

Epidemiological data suggest that obesity is linked with an increased risk of depression [37, 38]. Still, there is little understanding of the neural mechanisms and brain reward pathways

Table 2. Effect of ghrelin in treatment on oxidative stress in the brain of rats subjected to HFD and DDR

Treatment	MDA (μg/mg of protein)	GSH (μg/mg of protein)	CAT (U/mg of protein)	SOD (U/mg of protein)
ND+NR	19.89±1.9	37.06±2.11	32.96±2.07	35.27±1.75
SHAM+DDR	59.38±3.3	30.72±2.14	20.89±0.5	25.46±1.35
ND+DDR	21.94±1.9	32.70±1.59	27.64±1.3	28.56±1.11
HFD+NR	35.67±2.3	29.63±1.81	24.24±0.9	24.33±1.42
HFD+DDR	78.07±3.5#	18.12±1.09#	16.28±0.6#	21.12±0.96#
HFD+DDR+GHR	24.39±1.4*	36.89±2.62*	30.55±2.9*	34.13±0.91*
HFD+DDR+GHR+ANTA	26.15±0.8	34.50±2.13	33.09±2.9	30.53±1.51

The data were expressed as mean ± SEM and analyzed by using one-way ANOVA followed by Bonferroni's post hoc test. #P<0.001 vs. ND+NR; *P<0.001 vs. HFD+DDR. ANOVA, analysis of variance; ANTA, ghrelin antagonist; CAT, catalase; DDR, disturb diurnal rhythm; GHR, ghrelin; GSH, glutathione; HFD, high fat diet; MDA, malondialdehyde; ND, normal diet; NR, normal rhythm; SEM, standard error of the mean; SOD, superoxide dismutase.

Table 3. Effect of ghrelin treatment on cytokines levels in the brain of rats subjected to HFD and DDR

Treatments	TNF-α (pg/mg of protein)	IL-1β (pg/mg of protein)	IL-6 (pg/mg of protein)
ND+NR	16.86±0.7	115.4±3.9	14.15±1.2
SHAM+DDR	16.75±0.5	115.5±5.1	17.0±0.6
ND+DDR	23.66±2.5	123.2±2.6	13.53±0.4
HFD+NR	19.38±0.5	131.2±4.1	23.17±1.8
HFD+DDR	24.44±2.2#	164.2±9.2#	25.51±2.7#
HFD+DDR+GHR	15.30±0.7*	114.6±2.3*	12.91±0.9*
HFD+DDR+GHR+ANTA	15.45±0.9	116.3±1.6	14.77±1.4

The data were expressed as mean ± SEM and analyzed by using one-way ANOVA followed by Bonferroni's post hoc test. #P<0.001 vs. ND+NR; *P<0.001 vs. HFD+DDR. ANOVA, analysis of variance; ANTA, ghrelin antagonist; DDR, disturb diurnal rhythm; GHR, ghrelin; HFD, high fat diet; IL, interleukin; ND, normal diet; NR, normal rhythm; SEM, standard error of the mean; TNF, tumour necrosis factor.

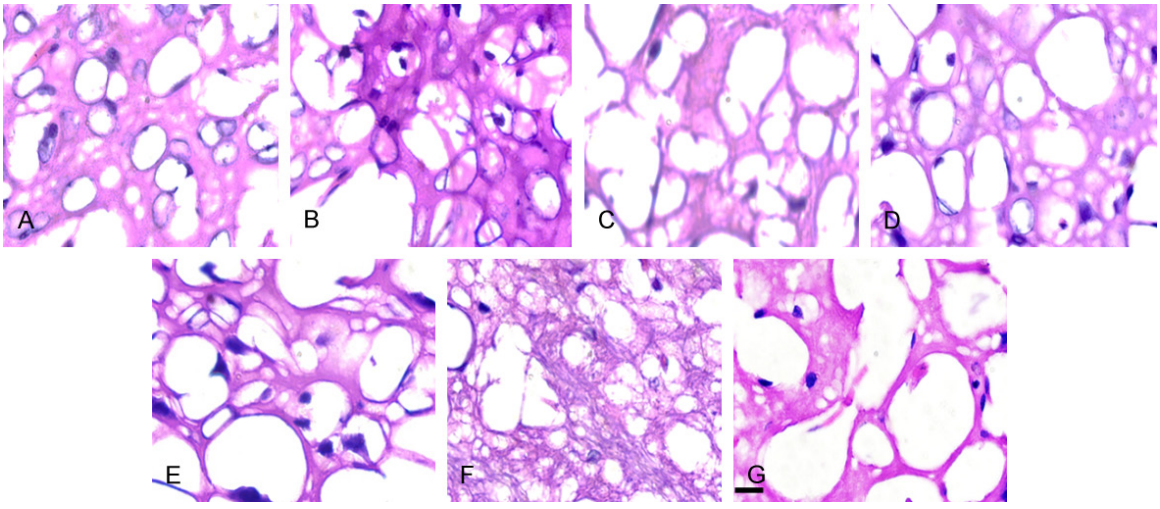

Figure 4. Effect of ghrelin treatment on histopathology of brain of rats subjected to HFD and DDR. A. ND+NR; B. SHAM+DDR; C. ND+DDR; D. HFD+NR; E. HFD+DDR; F. HFD+DDR+GHR; G. HFD+DDR+GHR+ANTA. ANTA, ghrelin antagonist; DDR, disturb diurnal rhythm; GHR, ghrelin; HFD, high-fat diet; ND, normal diet; NR, normal rhythm. Scale bar =100 μ.

that underlie the link between diet-induced obesity and vulnerability to depression. In the present study, we found that chronic consumption of HFD significantly increased the body

weight, and induced several biochemical modifications in brain reward circuitry which might lead to depressive-like behavior. This is in line with earlier study [16], which showed that HFD consumption, besides inducing obesity, can also alter the animal's behavioral state by promoting depressive- and anxiety-like behaviors. Another previous study demonstrated a link between the phenomena of depressive disorders and diurnal rhythms [39]. In the present study, we demonstrated that diurnal rhythm disturbance for 45 days results in depressive behavior [6].

In FST, the HFD+DDR group showed higher immobility time as compared to the normal group. Treatment with ghrelin showed a decrease in immobility time which indicates decrease in their depressive behavior. FST is well known to monitor depressive-like behavior and is based on the assumption that immobility reflects a measure of behavioral despair [40]. In OFT, the HFD+DDR animals showed higher immobility time and a smaller number of line crossings. Treatment with ghrelin showed a decrease in immobility time in the FST with an increase in mobility time in the OFT, which confirms ghrelin as a potential molecule to reduce depression. A prominent reduction in closed arm entries was demonstrated as anti-depressive behavior [41]. An identical study was performed previously mentioning a decrease in closed arm entries as a marker of antidepressant behavior [42]. In EPM, before the treatment, the HFD+DDR group animals showed an increase in the number of entries as well as time spent in the closed arm. Treatment with the ghrelin showed the maximum entries and time spent in the open arm. This suggests that ghrelin treatment in HFD+DDR exposed rats showed a decrease in depression-related phenotype characters. Further, EPM results indicate the role of ghrelin in spatial learning and memory. In the rotarod test, the animals showed immediate latency to fall in the HFD+DDR group as compared to the normal group before treatment, results were reversed after treatment with the ghrelin. It showed improvement in motor activity of animals specifying the role of ghrelin against depression.

The SOD activity and MDA level, are changed in major depression as reported previously [43]. Enhanced oxidative stress was observed in the chronic stress-induced depression in rats, mainly expressed as the significant increase of MDA level and the significant decrease of SOD and CAT activity [44]. Extensive production of reactive oxygen species is the sign of activation of lipid peroxidation in biological membranes and their overproduction causes loss of fluidity in cell membranes [45]. In the present study, we estimated the levels of antioxidants like MDA, GSH, SOD and CAT in brain homogenates. The antioxidative enzymes like CAT and SOD regulate the redox status through counteracting free radicals [46]. The levels of GSH, SOD and CAT were depleted, correlating to the compromised antioxidant mechanism of brain tissue resulting from exposure to HFD and DDR. The major relevance of an increment in oxidative stress is lipid peroxidation (oxidation of lipids by free radicals catalyzed by peroxidases). The end product of lipid peroxidation is MDA. It reacts with cells and proteins or DNA to form adducts and induces bimolecular damage. In the current study, ghrelin promisingly depleted the levels of thiobarbituric acid reactive substances, indicating a significant decrease in lipid peroxidation.

The pro-inflammatory cytokines have been stated to have a role in depression, such as IL-1β and TNF-α [47]. In the central nervous system (CNS), IL-1β may stimulate the production of other cytokines (such as IL-6 or TNF-α) by astrocytes and microglia, hence promoting inflammatory processes in the brain [48]. Therefore, the effects of interactions among cytokines in the CNS on the etiology of depression cannot be ignored. In addition, in view of the HPA (hypothalamus-pituitary-adrenal) axis theory of depression, the cytokines also played roles in mediating the activity of the HPA axis. The previous studies showed that IL-1β or IL-6 is involved in the hyperactivity of the HPA axis [48-50]. However, the IL-6 signalling pathway involved in the etiology of depression has rarely been demonstrated. In the present study, TNF-α, IL-1β and IL-6 levels were relatively high in the HFD+DDR as compared to the control group. Ghrelin efficiently reduces the levels of pro-inflammatory cytokines. Further, the histopathological study confirms the role of ghrelin in alleviating the depressive behavior in the rats as evidenced by a reduction in the cell infiltration and edema in the rats treated with the ghrelin. While HFD+DDR showed a significant increase in neutrophil infiltration, edema, and severe necrosis suggesting neuronal damage.

Conclusion

Ghrelin showed a significant change in behavioral paradigms as well as improving the endogenous antioxidant levels complying with overall improved neuronal functions. Furthermore, the arrest in pro-inflammatory levels indicated enhanced neuronal protection. Future studies are required to be carried out to understand the mechanism behind the neuroprotective ability of ghrelin.

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Disclosure of conflict of interest

None.

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