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Potential therapeutic effect of teneligliptin on unpredictable chronic mild stress-induced depression in mice: targeting the role of AMP-activated protein kinase signaling pathway

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Background
Depression is a widespread and complex mental disorder that is challenging to treat. The unpredictable chronic mild stress (UCMS) model, based on stress diathesis, is used to study depression because antidepressants often reverse its effects, providing predictive validity. However, the mechanisms behind UCMS are not fully understood. AMP-activated protein kinase (AMPK) is essential for regulating neuronal energy metabolism, and disruptions in its activation can impair brain function and synaptic integrity. Teneligliptin, a dipeptidyl peptidase-4 (DPP-4) inhibitor with anti-inflammatory, antioxidant, autophagy-modulating, and antiapoptotic properties, has not been studied for its potential to activate AMPK by inhibiting DPP-4, stimulating glucagon-like peptide-1, and decreasing the mammalian target of rapamycin pathway, which may enhance neuroprotection and neurogenesis. This study is the first to show teneligliptin's potential therapeutic effect on UCMS-induced depression in mice by targeting the AMPK signaling pathway.

Objective
The current study was performed to assess the behavioral, oxidative stress biomarkers, neurotransmitter assay, and histopathology of the brain in UCMS-induced depression in mice.

Materials and methods
Mice were divided into five groups: group I (normal control, received saline), group II (UCMS model with 1 week of stress), group III (UCMS+fluoxetine 10 mg/kg daily for 4 weeks), and groups IV and V (UCMS+teneligliptin 30 mg/kg and 60 mg/kg, respectively, for 4 weeks). Body weight, forced swim test, tail suspension, locomotor activity, oxidative biomarkers (malondialdehyde, reduced glutathione, superoxide dismutase, nitrite levels), neurotransmitters (dopamine, serotonin), and brain histopathology were assessed to evaluate antidepressant activity.

Results and conclusion
Mice treated with teneligliptin (30 and 60 mg/kg) in the UCMS model showed significant weight loss compared with the disease group. They also exhibited reduced immobility in forced swim and tail suspension tests and increased locomotor activity. Significant changes were noted in oxidative stress biomarkers and neurotransmitters (dopamine, serotonin), with improved histopathological conditions. In conclusion, teneligliptin improved behavioral and antidepressant effects by reducing oxidative stress and increasing dopamine and serotonin levels. As a DPP-4 inhibitor, it may activate AMPK in the brain, lower DPP-4 enzyme levels, and enhance glucagon-like peptide-1-R and mammalian target of rapamycin, potentially promoting neurogenesis and neuroprotection, thus being beneficial for depression treatment.

Keywords:
AMP-activated protein kinase, depression, dipeptidyl peptidase-4 inhibitor, unpredictable chronic mild stress model

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Introduction

Depressive disorders are marked by poor mood, anhedonia, negativity, delayed words, and delayed actions. At the moment, depression affects more than 300 million people globally and is the most prevalent cause of morbidity [1]. While there are numerous clinically useful antidepressant drugs, the majority of them have detrimental side effects. The three main signs of the psychopathological condition are anhedonia, low energy or weariness, and low or sad mood. Additional signs are frequently present as well,

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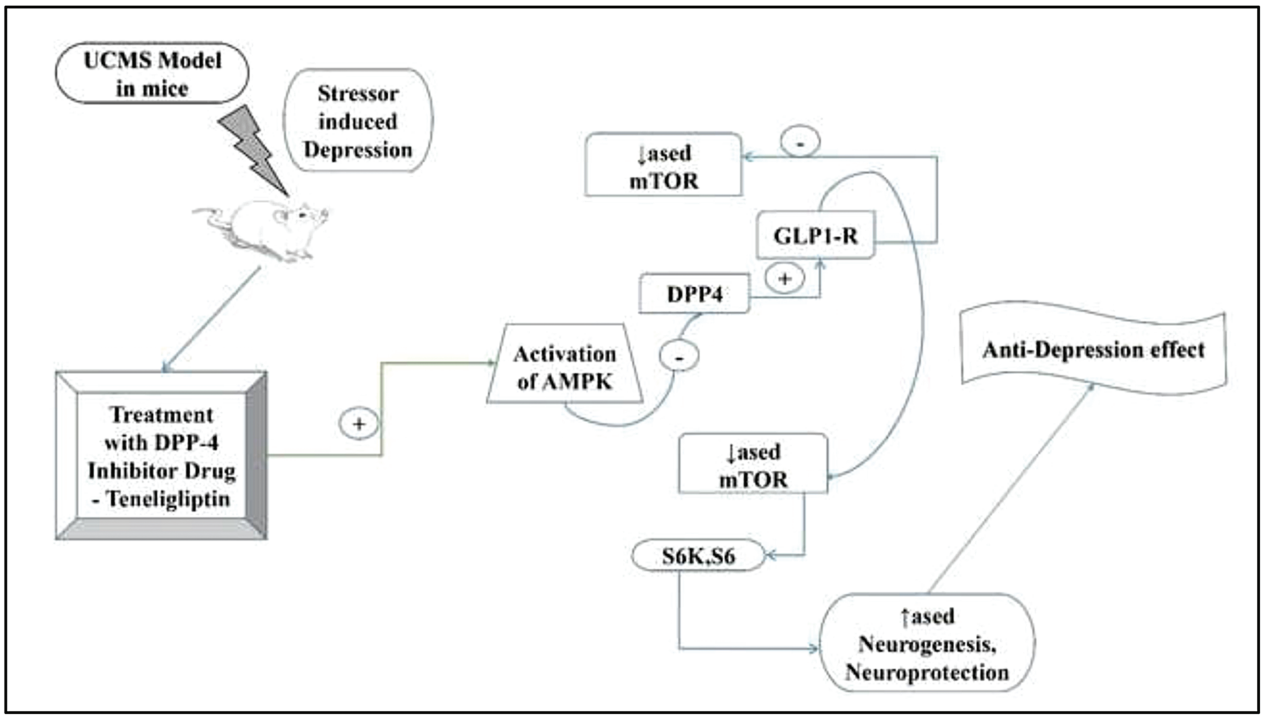
including disturbed sleep and psychomotor functions, guilty feelings, feelings of worthlessness, thoughts of suicide and sentiments, and autonomic and gastrointestinal problems. As a result, depression has a severe negative influence on patients' lives and places a great strain on families and society [2]. The pathophysiology of depression is closely linked to damaged neurogenesis, abnormally high stress levels, and diminished synaptic plasticity. The prefrontal cortex, hippocampus, amygdala, and nucleus accumbens are among the limbic brain regions that have been linked to the mediation of important signs related to depression as well as anxiety. Microglial cells are the central nervous system's macrophages. It has been shown that stimulation of these cells in the hippocampal region can promote the secretion of pro-inflammatory factors, which can disrupt neuroplasticity, impair cognitive function, and ultimately exacerbate depression [3,4]. AMP-activated protein kinase (AMPK) signaling pathway has been identified to regulate depressive-like behaviors in an unpredictable chronic mild stress (UCMS) animal model [5]. AMPK has the potential to negatively impact the protein complex known as nuclear factor kappa-light-chain enhancer of activated B cells. Thus, by regulating the production as well as stimulation of its downstream components, AMPK, a highly conserved serine/threonine protein kinase, affects the enzyme's redox balancing, regulates catabolism and anabolism, and has anti-inflammatory properties [6]. A vital role for metabolite-sensing protein kinase, AMPK, is its ability to sense changes in the intracellular ratio of AMP to ATP and controlling the processes of glycolysis and fatty acid oxidation. It belongs to a αβγ heterotrimeric that is phosphorylated on threonine 172 to activate. Numerous significant roles for AMPK have been linked to the brain's control of the circadian rhythm, nutrition, memory, learning, and synaptic plasticity. AMPK activation has been associated with increased neurogenesis, the process by which new neurons are generated in the brain. This could potentially help repair or replenish brain regions affected by depression. The exact mechanisms through which AMPK promotes neurogenesis are still being investigated but may involve the activation of signaling pathways that support neuronal growth and survival. AMPK activation has been shown to have anti-inflammatory effects by inhibiting the activity of pro-inflammatory signaling pathways and promoting the production of anti-inflammatory molecules. By reducing neuroinflammation, AMPK activation may help lighten some of the symptoms of depression. AMPK may influence the levels and activity of neurotransmitters such as serotonin, dopamine, and norepinephrine, which are known to play key roles in mood regulation. AMPK activation can enhance mitochondrial function, leading to increased energy production within cells. Improving mitochondrial function may enhance neuronal resilience to stress and promote overall brain health, potentially reducing the risk of depression [7–9].

Dipeptidyl peptidase-4 (DPP-4) inhibitors, or DPP-4 activator medicines, are a class of pharmaceuticals that are frequently used to treat type 2 diabetes. They function by preventing the breakdown of incretin hormones such as glucagon-like peptide 1 (GLP1) and glucose-dependent insulinotropic peptide by the enzyme DPP-4. These drugs prolong the effects of GLP1 and glucose-dependent insulinotropic peptide by blocking DPP-4. This causes an increase in insulin production and a decrease in glucagon release, which in turn lowers blood sugar levels [10]. One important cellular energy sensor that controls energy metabolism is called AMPK. AMPK is triggered in situations where there is a decrease in cellular energy, such as during exercise or fasting. AMPK activation triggers a range of metabolic reactions that work to restore energy balance. These reactions include enhanced absorption of glucose, fatty acid oxidation, and mitochondrial biogenesis, as well as inhibition of energy-consuming processes [11]. DPP-4 inhibitors can reduce oxidative stress and inflammation in the brain, which may have neuroprotective effects. As AMPK may control cellular energy homeostasis and lessen oxidative stress, it has been linked to neuroprotection. DPP-4 inhibitors may therefore indirectly activate AMPK in the brain, producing neuroprotective benefits that may be pertinent to depression [12]. It has been demonstrated that DPP-4 inhibitors raise the brain's concentrations of neurotrophic factors such as brain-derived neurotrophic factor. Brain-derived neurotrophic factor is essential for mood control, synaptic plasticity, and neuronal survival. DPP-4 inhibitor drugs that suppress DPP-4 are crucial for the stimulation of AMPK. AMPK activation decreases the DPP-4 enzyme and improves GLP1-R, which controls mammalian target of rapamycin (mTOR). Reduced mTOR levels may enhance neurogenesis and neuroprotection, normalize programmed apoptosis, which is beneficial for depression and its symptoms at a low level (Fig. 1). AMPK has been demonstrated to control the brain's dopamine and serotonin signaling pathways. DPP-4 inhibitors may therefore indirectly affect these neurotransmitter systems through AMPK activation, which may be a factor in the antidepressant effects of the drug.

Figure 1



Graphical presentation of DPP-4 inhibitor drug teneligliptin on depression. ↑, increased level; ↓, decreased level; AMPK, AMP-activated protein kinase; DPP-4 inhibitor, dipeptidyl peptidase-4 inhibitor; GLP1, glucagon-like peptide-1; mTOR, mammalian target of rapamycin; UCMS model, unpredictable chronic mild stress model.

Materials and methods

Drugs and chemicals

Teneligliptin was received as a gratis sample from Ami Lifesciences Pvt. Ltd. in Vadodara, India. Fluoxetine was received as a gratis sample from Palam Pharma Pvt. Ltd in Ahmedabad, India. All the analytical grade chemicals and other reagents used in the experiment were supplied by Sandeep Organics Pvt. Ltd. India.

Animals and housing

Male Swiss albino mice with a regular weight of 25±2 g and 7–8 weeks old were obtained from the Zydus Research Centre located in Ahmedabad, which were then accommodated at the Sumandeep Vidyapeeth Deemed to be University, Department of Pharmacy. Typical conditions, such as a 12-h light and dark cycle, 22±5°C temperature, and regulated humidity at 55 ±5%, were maintained in the animal facility. The Institutional Animal Ethics Committee (IAEC) of Sumandeep Vidyapeeth permitted all experimental techniques defined in this work. The University of Vadodara's Department of Pharmacy is eligible under the standards of the Ministry of Social Justice and Empowerment, the Government of India's Committee for Control and Supervision of Experiments on Animals (CCSEA) Protocol number SVU/DP/IAEC/2022/11/59 was adhered to during the experimentation process. Before the initiation of the experiment, the mice were given a week to adjust to their new environment.

Experimental design

The mice were allocated randomly into five groups, as outlined in Table 1. The treatment regimen spanned a duration of 4 weeks. The animals' weights were recorded on the first day of the experiment so that the dosages could be calculated. All medication solutions were prepared by dissolving them in normal saline, and dose selection was done according to previous research. The different stressor approach was used to generate depression as per

Table 1 Animal group design protocol

Groups		Animals (mice)
Group I	Normal control group (0.5% w/v saline)	6
Group II	Unpredictable chronic mild stress model for 7 days	6
Group III	Unpredictable chronic mild stress model for 7 days followed by 10 mg/kg fluoxetine for 28 days (PO)	6
Group IV	Unpredictable chronic mild stress model for 7 days followed by 30 mg/kg teneligliptin for 28 days (PO)	6
Group V	Unpredictable chronic mild stress model for 7 days followed by 60 mg/kg teneligliptin for 28 days (PO)	6

Table 2 Details of stressor procedure

Days	Morning	Afternoon	Evening
Monday	Vibration of the cage	Wet pens	Swimming in cold water
Tuesday	Combining	Sloping cage at a 45-deg angle	Constraint
Wednesday	Dry heat	Constraint	Dumping of bedding
Thursday	Lack of food and water	–	–
Friday	Vibration of the cage	Sloping cage at a 45-degree angle	Swimming in cold water
Saturday	Wet pens	Restrained motion	Constraint
Sunday	Pairing	Dry heat	Dumping of bedding

UCMS model-induced depressive symptoms in mice [13]. Stressors included deprivation of food and water for a full day, cages sloping 45°, vibration exposure, swimming in 4° water, bedding deprivation for 16 h, 6 h in a wet cage, 2 h of restrained motion, 2 h of constraint, and 2 h of pairing. Table 2 presents the specifics of the stressors for a week. Following the conclusion of the stressor exposure, behavioral assessments were conducted to confirm the presence of depression. After the 4 weeks of treatment period, animals underwent evaluations encompassing behavioral analyses, biochemical and oxidative stress assessments, neurotransmitter levels, and histopathological examinations. Brain samples were collected to measure neurotransmitters, oxidative stress parameters, and histopathology of the brain. Blood samples were obtained on the 36th day; the retro-orbital area was punctured under light anesthesia, and the samples were then stored in tubes containing EDTA. The serum was extracted by centrifuging of the blood for 20 min at 5000 rpm after it had clothed for 15 min, the resultant serum was kept at −20°C. For the evaluation of antidepressant activity, that is, body weight, behavioral parameters including forced swim test (FST), tail suspension test (TST), and locomotor activity measurement for stress-induced behavioral parameters were chosen. Neurotransmitter assay of dopamine and serotonin levels were done. Oxidative stress parameters include analysis of malondialdehyde, reduced glutathione, superoxide dismutase, and nitrite levels, as well as histopathology of the brain.

Body weight

During the experiment, body weight was to be dignified and noted from the first day until the 36th day.

Behavioral parameters

The FST followed the Porsolt and colleagues method, where mice were placed in a cylindrical water tank (70 cm high, 40 cm diameter) with water at 23–25°C for 5 min. A 15-min pretest occurred 24 h earlier. Swimming and immobility times were recorded using a stopwatch. The water was changed after each test, and the mice were dried in a heated enclosure [14].

TST: in this test, the mice were subjected to unavoidable stress by hanging them by their tails, which caused them depressive symptoms such as immobile posture and a decrease in escaping tendency. Tail suspension tests are intended to evaluate the potency of various antidepressant agents. This test measures the duration of immobility, which indicates a state of despair when subjected to unavoidable stress, which reflects human depressive disorders. In this model, the mice were suspended from a platform at a height of 50 cm above the ground with the help of adhesive tape, which was placed 1 cm above the tip of the tail. The duration of immobility was recorded for the last 4 min for a total of 6 min of suspending time [15].

Locomotor behavior: locomotor activity serves as an indicator of animal behavior. The actophotometer was used to monitor the locomotor behavior of mice. Each mouse was placed individually into the actophotometer, where the fundamental concept involves the animals moving from one side to another, passing through a beam, and the readings being displayed. The baseline activity score was recorded by the actophotometer over a period of 3 min [16,17].

Determination of oxidative stress biomarkers and neurotransmitter levels

One day following the last treatment, the animals were killed by a higher dose of anesthesia [18], and the brain of each mice was immediately dissected out, washed with ice-cooled physiological saline, and homogenized in 0.15 M potassium chloride solution. Aliquots of the homogenate (20%) were prepared for the assessment of brain contents of malondialdehyde, reduced glutathione, superoxide dismutase (SOD), and nitrite level using previous researcher's methods [19]. The neurotransmitter level of dopamine and serotonin was determined using the method described by Nazir *et al.* [18].

Histopathology of the cortex and the hippocampus region of the brain

At the end of the experiment, tissue samples for histology were fixed in a 10% formalin solution for 24 h. They were then washed with water, passed through ascending grades of ethanol, cleared with xylene, and embedded in paraffin wax. The paraffin blocks were trimmed, cut into 4 µm thick sections, mounted on slides, and stained with hematoxylin and eosin (×40). All sections were examined using an Olympus light microscope [18].

Statistical analysis

The mean±SEM was the intended method of presenting the data. Following a one-way analysis of variance, posttests were conducted using a computer-based fitting program (Graph Pad Prism 8.0) to identify any significant differences through multiple comparisons. To determine the statistical significance, we used a significance level of *P* value less than 0.05.

Results

Effect of teneligliptin on body weight

The normal control group (27.50±0.46) compared with the animals' weight increased significantly ($P<0.05$) throughout the disease group (31.40±0.73) of UCMS. The reduction of body weight of mice was observed which was significant ($P<0.05$) after the start of the treatment group of teneligliptin, which is 30 mg/kg (26.95±0.59) and 60 mg/kg (25.45±0.57) in comparison with the disease group of UCMS (Fig. 2).

Effect of teneligliptin on behavioral parameters

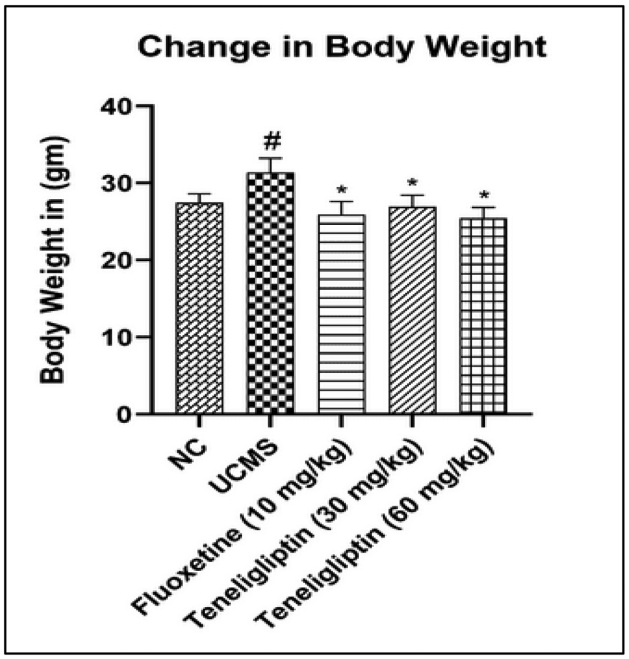
Force swim test

The disease group of UCMS (62.17±1.51) had a significantly longer period of immobility than a group of normal control group (30.67±1.54) ($P<0.05$). Comparing the disease groups to the treatment groups receiving teneligliptin – 30 mg/kg (46.33±2.53), 60 mg/kg (37.50±2.47), and a standard control group of fluoxetine – 10 mg/kg (42.67±1.58), a significant reduction in immobility time was observed ($P<0.05$) (Fig. 3).

Tail suspension test

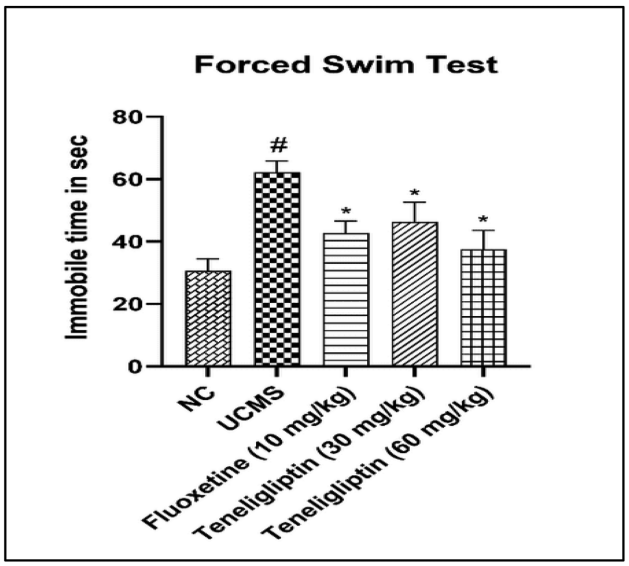
Animals' reduced range of motion indicates their immobility. In the current study, the disease group's UCMS immobility time increased (148.70±1.92) in a significant manner as compared with the group of normal control (94.17±6.18) ($P<0.05$). Treatment groups of teneligliptin – 30 mg/kg (123.0±2.98), 60 mg/kg (104.2±2.81), and standard control groups of fluoxetine – 10 mg/kg (123.7±2.45) have

Figure 2



Effect of teneligliptin on body weight in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated at doses of 30 and 60 mg/kg.

Figure 3



Effect on forced swim test by teneligliptin in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated with 30 and 60 mg/kg.

significantly ($P<0.05$) declined immobility times than the disease groups of UCMS (Fig. 4).

Locomotor activity

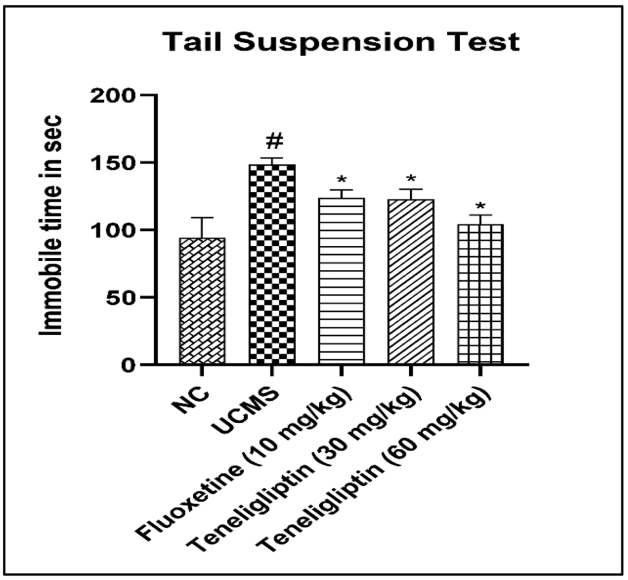
In comparing the disease group's UCMS (303.3 ±28.58) to the group of normal control (415.0 ±11.69), a declined locomotor activity was observed in a significant manner for the disease control group ($P<0.05$). The locomotor activity significantly increased ($P<0.05$) for all the treatment groups: teneligliptin at −30 mg/kg (378.3±13.33), 60 mg/kg (407.5±10.14), and standard fluoxetine at 10 mg/kg (373.3±22.57) in comparison with the disease control group (Fig. 5).

Effect of teneligliptin on parameters of oxidative stress

Level of malondialdehyde, reduced glutathione, superoxide dismutase, and nitrite

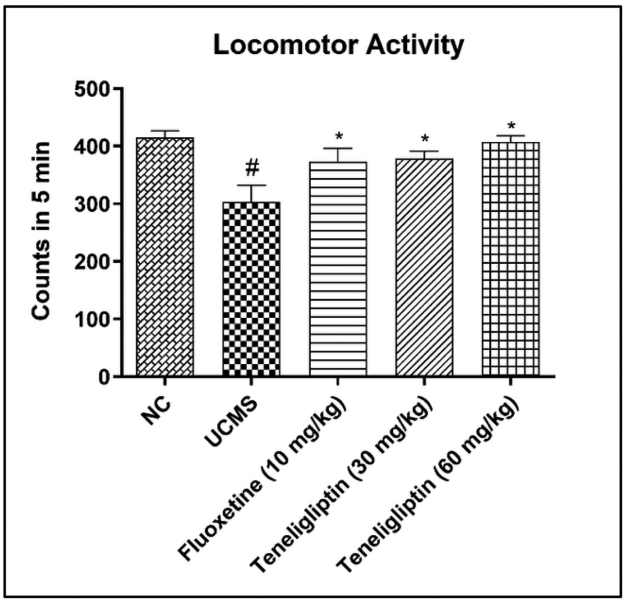
In comparison to the UCMS and group of normal control, a significant decline in the levels of malondialdehyde (13.84±0.62) and reduced glutathione (18.56±0.79) was observed. Comparing the groups receiving treatment, teneligliptin at 10 mg/kg (23.63±0.84), 60 mg/kg (29.86±0.67), and standard fluoxetine at 10 mg/kg (24.90±0.65) of the malondialdehyde level and glutathione level of the treatment group of teneligliptin at 30 mg/kg (23.25 ±0.78), 60 mg/kg (27.91±0.41), and standard

Figure 4



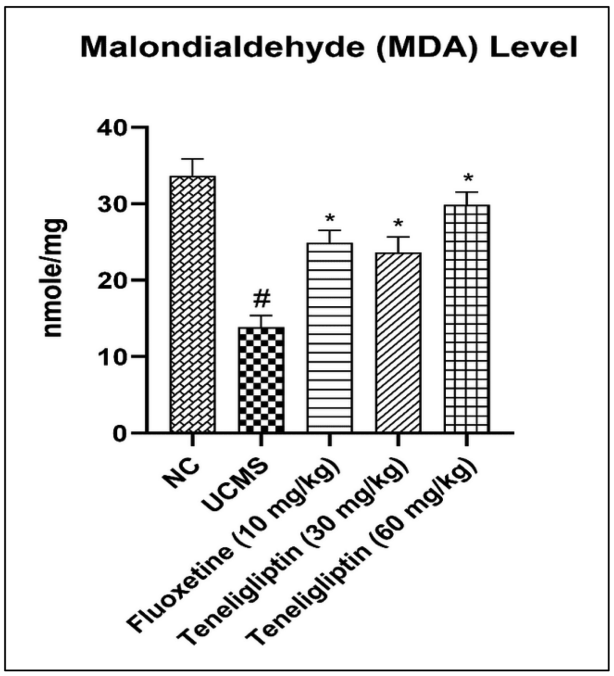
Effect on tail suspension test by teneligliptin in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated with 30 and 60 mg/kg.

Figure 5



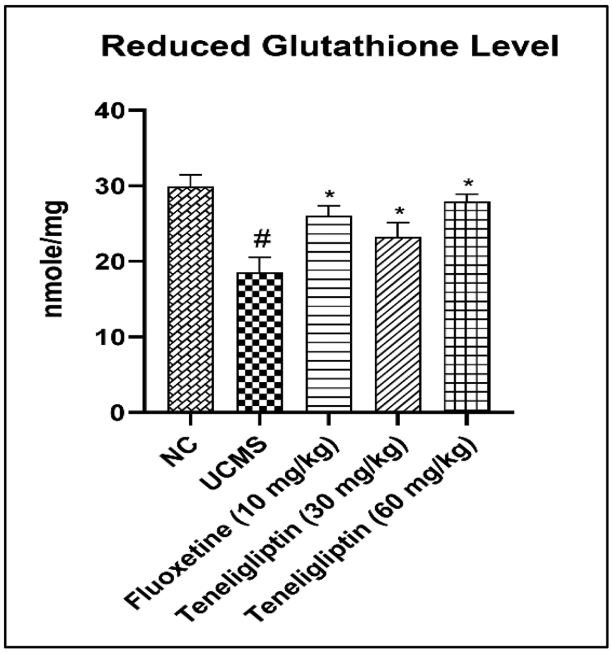
Effect on tail suspension test by teneligliptin in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC=normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated with 30 and 60 mg/kg.

Figure 6



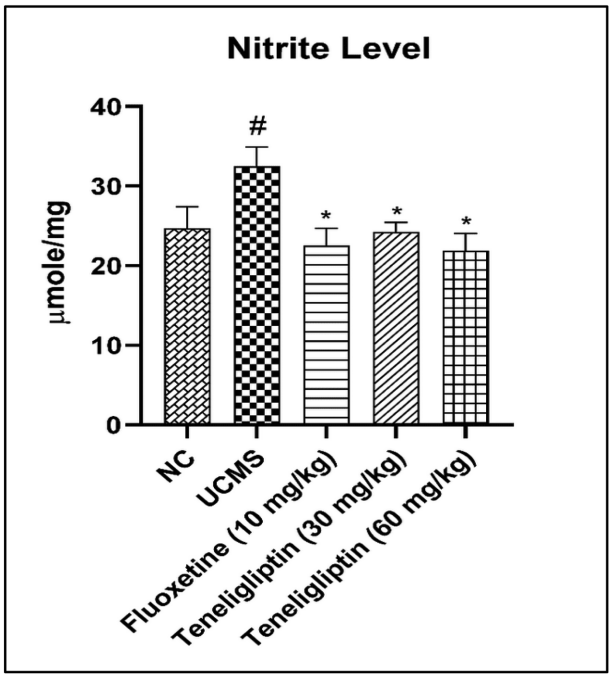
Effect of teneligliptin on malondialdehyde in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated with 30 and 60 mg/kg.

Figure 7



Effect of teneligliptin on reduced glutathione in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated with 30 and 60 mg/kg.

Figure 8



Effect of teneligliptin on nitrite in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS+teneligliptin treated with 30 and 60 mg/kg.

fluoxetine at 10 mg/kg (26.06±0.52) with the UCMS group the level of malondialdehyde (Fig. 6) and reduced glutathione (Fig. 7) was increased in a significant manner ($P<0.05$). In comparison to the UCMS and group of normal control SOD (14.68 ±1.04) and nitrite (24.17±1.09), a significant increase in the levels of nitrite (32.52±0.98) and superoxide dismutase (23.20±0.74) was observed. Comparing the SOD level of groups receiving treatment at 30 mg/kg (19.70±0.53), 60 mg/kg (17.93±0.71), and standard fluoxetine at 10 mg/kg (16.95±0.64) and nitrite level of groups receiving treatment at 30 mg/kg (24.20 ±0.50), 60 mg/kg (21.88±0.89) and standard fluoxetine at 10 mg/kg (22.53±0.88) with the UCMS group, the level of nitrite (Fig. 8) and superoxide dismutase (Fig. 9) was decreased in a significant manner ($P<0.05$).

Effect of teneligliptin on neurotransmitters

Dopamine level

Dopamine levels (µg/dl) in the UCMS group (0.097 ±0.004) were significantly lower than in the normal control group (0.142±0.005) ($P<0.05$). In comparing the groups receiving treatment group teneligliptin at 30 mg/kg (0.118±0.003), 60 mg/kg (0.131±0.005), and standard fluoxetine at 10 mg/kg (0.111±0.003) with the UCMS group, the level of dopamine was increased in a significant manner ($P<0.05$) (Fig. 10).

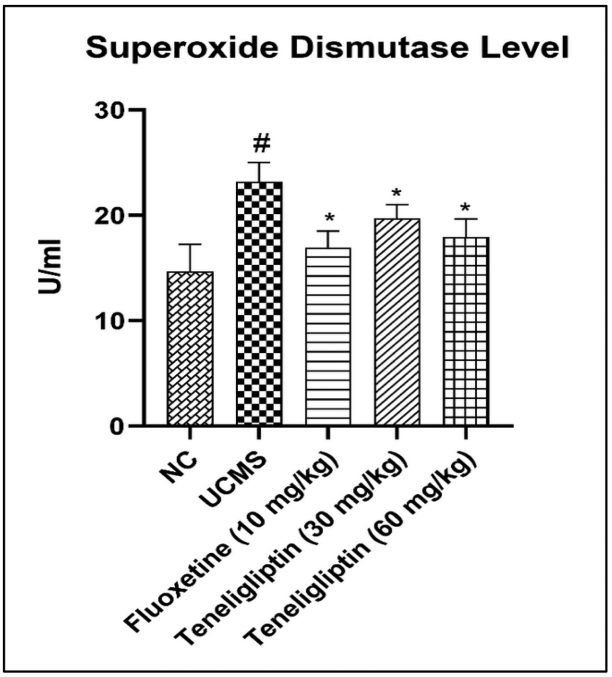
Serotonin level

Serotonin levels (µg/dl) in the UCMS group (0.084 ±0.003) were significantly decreased as compared with the normal control group (0.160±0.003) ($P<0.05$). In comparing the groups receiving treatment group teneligliptin at 30 mg/kg (0.118±0.003), 60 mg/kg (0.147±0.004), and standard fluoxetine at 10 mg/kg (0.145±0.003) with the UCMS group, the level of serotonin was increased in a significant manner ($P<0.05$) (Fig. 11).

Effect of teneligliptin on the evaluation of brain histopathology

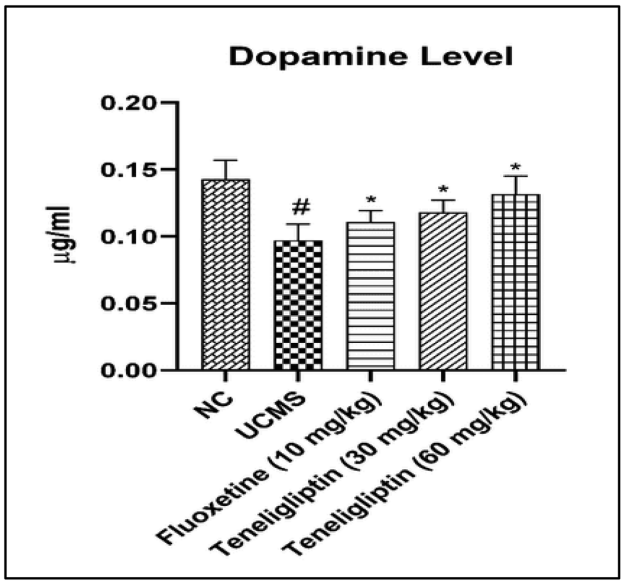
The normal control group shows a normal structure of neurons having enormous pale-stained nuclei, the nuclear chromatin, and well-known nuclei disappeared, and the adjacent support cells (glial cells) that have small nuclei with heavily stained, strong chromatin (Fig. 12a, the normal group). Microscopic examination of the cortical tissue in the UCMS model's disease group showed severe neural injury, including spongiosis, focal gliosis around degenerating neurons, size variations, vacuolization,

Figure 9



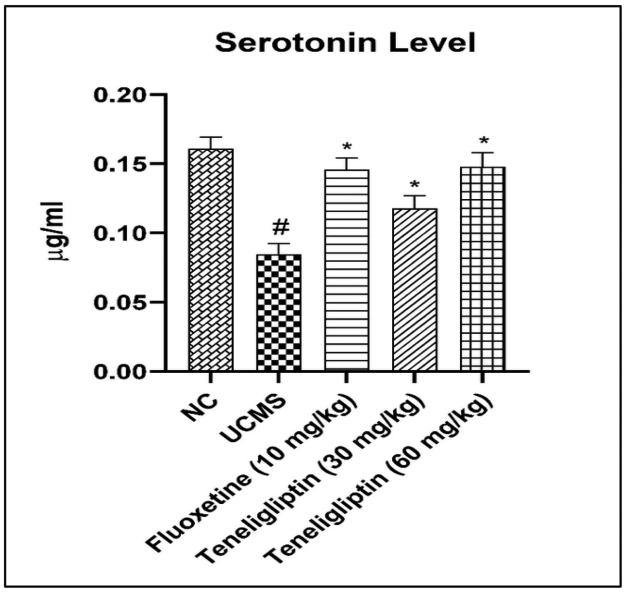
Effect of teneligliptin on superoxide dismutase in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS+teneligliptin treated with 30 and 60 mg/kg.

Figure 10



Effect of teneligliptin on dopamine in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated with 30 and 60 mg/kg.

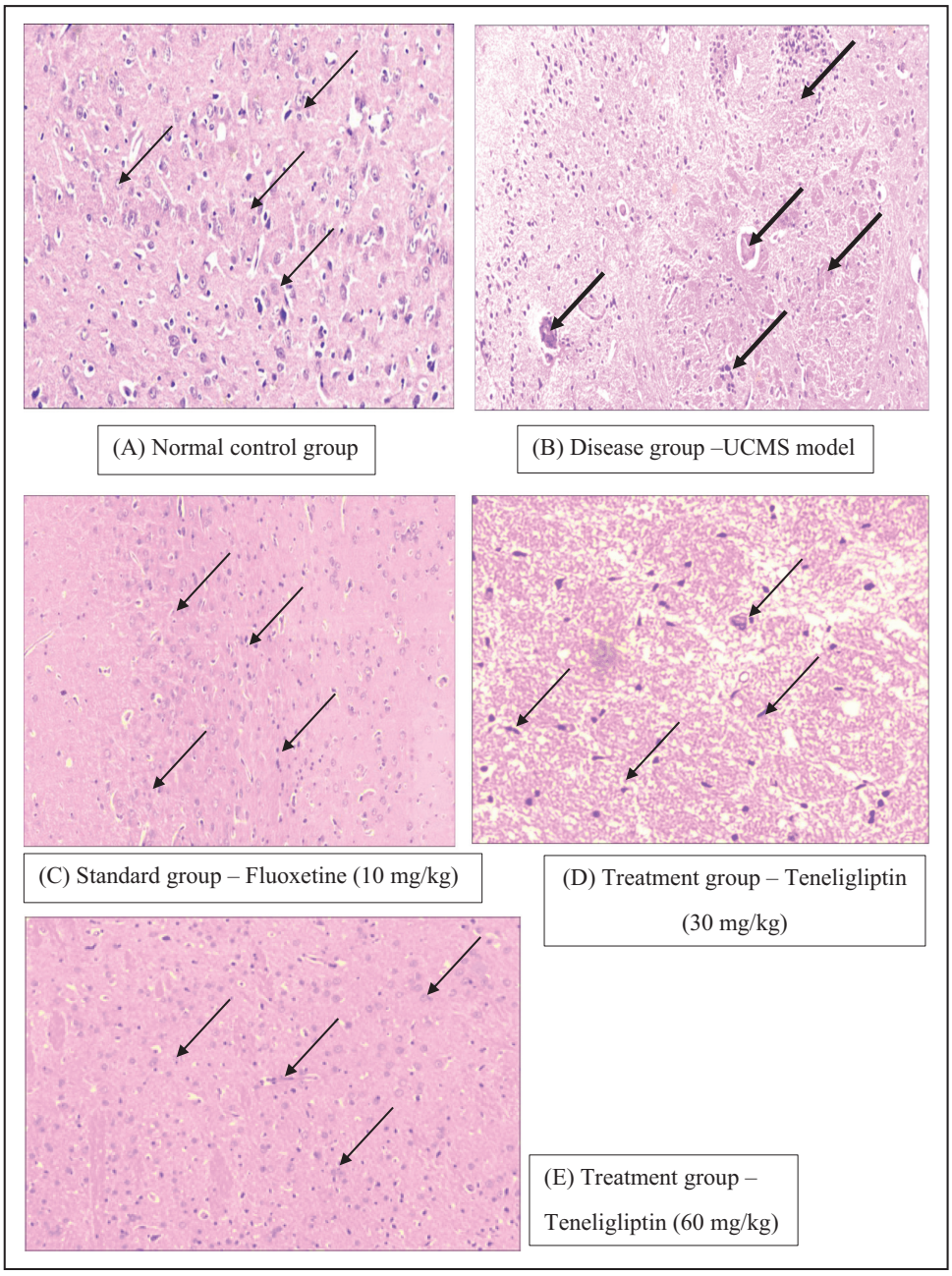
Figure 11



Effect of teneligliptin on serotonin in unpredictable chronic mild stress-induced mice model. There is a significant deviation from the normal control group ($^{\#}P<0.05$) and the disease control group ($^{*}P<0.05$). There are six animals in each group. Mean±SEM is used to express the values. NC, normal control group; UCMS, unpredictable chronic mild stress; UCMS+fluoxetine treated 10 mg/kg, UCMS +teneligliptin treated with 30 and 60 mg/kg.

shrinking, apoptosis, and lysis of neurons. (Fig. 12b, disease group, the UCMS model). In the case of administration of the standard group of fluoxetine 10 mg/kg drug, histopathological examination showed that the structure of neurons seemed more or less normal (Fig. 12c, standard group, fluoxetine 10 mg/kg). After treatment group with teneligliptin at doses of 30 and 60 mg/kg, the cortical tissue of the brain indicated nearly normal cortical tissue (Fig. 12d and e, treatment group 30 and 60 mg/kg). Normal control group showing a normal structure of neurons (arrow) having enormous pale-stained nuclei, the nuclear chromatin and well-known nuclei disappeared; the adjacent support cells (glial cells) (arrowhead) have small nuclei and heavily stained; disease group – microscopic examination of the cortical tissue in the UCMS model's disease group revealed severe neural injury, including spongiosis, focal gliosis around degenerating neurons, size variations, vacuolization, shrinking, apoptosis, and lysis of neurons; standard group – fluoxetine 10 mg/kg showing the structure of neurons that appeared more or less as normal; treatment group of teneligliptin drugs (30 mg/kg group) at low dose showed multiple vacuolated areas and pyknotic neurons; treatment group of teneligliptin drugs (60 mg/kg) group at high doses show nearly normal cortical tissue (hematoxylin and eosin, ×40).

Figure 12



Brain histology of unpredictable chronic mild stress induced in mice. Cross-section of mice brain in the (a) normal control group (×100, H & E), pyknotic cells are normally regular, and of stable nucleus, (b) disease group – pyknotic cells are characterized by irregular, shrunken, and damaged nuclei, while injured neurons exhibit variations in size, vacuolization, shrinking, apoptosis, and lysis, (c) standard group showing the structure of neurons that appeared more or less as normal, (d) treatment group – normalized pyknotic neurons and normal cortical tissue (→arrow indicates that the normal pyknotic cells are normally regular and of stable nucleus; →dark arrow indicates irregular, shrunken, and damaged nuclei of pyknotic cells).

Discussion

Depression is a crippling psychiatric condition that significantly impacts patients' quality of life, affecting millions worldwide. A challenge in treating depression is the absence of a specific target that provides effective long-term antidepressant effects. Depression is associated with the dysregulation of the hypothalamic–pituitary–adrenal (HPA) axis due to varied types of stress. The HPA axis is impacted by AMPK activation as well. The HPA axis decreased stress tolerance. The hormone responsible for the release of corticotrophin is secreted from the hypothalamus and binds to corticotrophin-releasing hormone receptors in the pituitary gland to trigger the HPA axis response. When this receptor interacts with the adrenocorticotropic hormone receptor on the adrenal gland, adrenocorticotropic hormone is

released. When this receptor is activated, cortisol is released, and cortisol regulates blood sugar, metabolism, memory formation, inflammation, and regulation. Numerous studies indicate that the AMPK activator is important in the pathophysiology of depression [20]. AMPK activator phosphorylates AMPK through an upstream route. By phosphorylating Bcl2, AMPK phosphorylation triggers the PI3K/Akt pathway, which promotes survival and inhibits apoptosis. In addition, mTOR, a regulator of the autophagy process, is modulated by PI3K/Akt. AMPK activation on the alpha-subunit causes the phosphorylation of the unc-51-like kinase 1 pathway, and an increase in AMP/ATP levels causes the autophagy process to be stimulated. Xeroderma pigmentosum, complementation group C and tuberous sclerosis complex 1 and 2 (TSC1/2) pathways are activated by AMPK activation on the γ subunit, and TSC1/2 promotes mTOR to promote protein synthesis and cell growth while reducing symptoms of depression [21,22]. An increasing amount of research indicates a connection between diabetes and stress progression. In laboratories, animal models such as UCMS and the isolation of social development are frequently used to simulate the onset of human depression.

Using a UCMS model, mice were given 1 week of different stressors. Numerous studies have shown that chronic stress produces weight gain because it increases food intake. In addition, UCMS groups have been shown to further increase food intake [23]. The body weight of the disease groups of mice in the current study increased significantly during disease induction, but it decreased significantly following treatment groups and the standard control group. To assess the depression-like behavior in mice, three parameters, namely, the FST, TST, and locomotor activity were used, which show behavioral despair and social fear in experimental animals. These tests confirmed depression in the UCMS group, evidenced by a significant increase in immobility time in both the FST and TST, further decreasing the locomotor count [24]. In this investigation, the groups treated with teneligliptin and standard fluoxetine demonstrated a significant decrease in immobility time in the FST and TST, along with a notable increase in locomotor activity compared with the disease control group. The study's behavioral characteristics showed that teneligliptin was as effective as a standard control group of fluoxetine at reducing depressive symptoms. The brain's antioxidant activity has been demonstrated to decline under conditions of constraint stress, according to numerous research. When the HPA axis is triggered by stress, leading to the production of reactive oxygen species, the adrenal gland releases glucocorticoids. Because endogenous antioxidants are known to express themselves rather poorly in neurons, oxidative stress can harm them greatly. Between the production of healthy oxidants and harmful oxidative stress, antioxidants establish an equilibrium. The observations reveal that the levels of malondialdehyde and reduced glutathione antioxidant enzymes are decreased significantly in the disease group (UCMS) [25]. The treatment groups of teneligliptin and standard fluoxetine ameliorate decreased levels of antioxidant enzymes. In addition, the nitrite and superoxide dismutase levels were increased in diseased groups, and after being treated with teneligliptin and standard fluoxetine showed a significant reduction in nitrite and superoxide dismutase levels. These findings indicate that therapies are keeping the redox state in balance. Synapses between neurons and target cells are facilitated by endogenous chemicals called neurotransmitters. Major excitatory neurotransmitters in the brain and spinal cord are dopamine and serotonin; these specific neurotransmitters are chiefly responsible for a multiplicity of diseases, which include anxiety, epilepsy, schizophrenia, and other common neurological disorders. Dopamine regulates the actions of other monoamines and serves as a neurotransmitter and neuromodulator inside the brain. Dopamine levels fall together with dopamine transporter levels in depressed conditions. Anxiety, mood, appetite, temperature, eating habits, sexual behavior, and movement are all influenced by the monoamine neurotransmitter serotonin [26]. Furthermore, it has been shown that insulin resistance causes behavioral disorders by changing the way serotonin is converted in the brain. In the current investigation, the UCMS group significantly decreased dopamine and serotonin levels. After the treatment, it was observed that dopamine and serotonin levels were increased in a significant manner. These findings suggest that the treatments reduce dysfunction related to dopamine and serotonin turnover in the brain. Important brain regions for learning and memory processes are the prefrontal cortex, the hypothalamus, and the hippocampal regions. Small nuclei in the hypothalamus carry out a variety of tasks. Through the pituitary gland, it connects the endocrine and neurological systems. The hormones secreted by the pituitary gland are either stimulated or inhibited by the hypothalamus hormones. According to earlier research, depression

modifies the morphology of the hippocampus, which changes how the structure operates. The brain's hypothalamus region histopathological analysis indicates that the disease group's UCMS of brain histopathology shows some morphological alterations and neurons were destroyed. Imbalance in the synaptic structural plasticity of all the given specific three regions of the brain has been associated with depression in animal models and human patients. The above given three regions of the brain in depressive patients possess atrophy significantly due to reasons such as reduced dendritic branches and their length, as well as spinal density of hippocampus neurons [18,27]. Depressed people tend to have smaller hippocampus size, which is linked with the harshness of their depression. Previous animal studies reveal that persistent stress induces prefrontal cortex and hippocampal dendritic shrinkage and neuronal death, causing depression-like behaviors. Normal nerve cells are shown by arrows. Pyknotic cells are irregular, shrunken, and have a damaged nucleus. Pyknosis is a condition that affects senescent (old) leukocytes and is caused by programmed cell death (apoptosis). Pyknosis is characterized by a thick, compact nucleus that fragments (karyorrhexis), resulting in the appearance of dark-staining nuclear chromatin spheres, indicated by dark arrows (hematoxylin and eosin, ×10). The majority of the histopathological data point to the beneficial effects of teneligliptin treatment doses. Our research revealed that teneligliptin enhances brain function in the regions such as the hippocampus, hypothalamus, and the prefrontal cortex. Our study suggests the activation of the AMPK by teneligliptin drug in the prefrontal cortex, hippocampus, and the hypothalamus region of the brain in a dose-dependent manner.

Conclusion

In conclusion, our results show that UCMS impairs cortical synaptic functioning and causes anxiety/depression. The UCMS model inactivates AMPK subunits α, β, γ for energy control. Different stressors of the UCMS paradigm may inactivate the AMPK pathway, which may contribute to depression. DPP-4 inhibitor drug of teneligliptin enhanced stimulation of the AMPK signaling pathway of subunits α, β, γ phosphorylation. Specifically, phosphorylation of α-subunits activates unc-51-like kinase 1 phosphorylation to increase autophagy, which will be beneficial in antidepression treatment. γ-subunit phosphorylation causes activation of TSC1/2 phosphorylation to activate the mTOR pathway, which in turn stimulates cell growth and protein synthesis in neuronal cells, and also inhibits apoptosis which proved beneficial in the treatment of depression. This research implies that the DPP-4 inhibitor drug of teneligliptin works through the mechanism of AMPK pathway activation and may be useful in addressing the pathological problems associated and therapy for better management of depression disorder.

AMPK, AMP-activated protein kinase; DPP-4 inhibitor, dipeptidyl peptidase-4 inhibitor; FST, forced swim test; GLP1, glucagon-like peptide-1; HPA, hypothalamic–pituitary–adrenal axis; mTOR, mammalian target of rapamycin; SOD, superoxide dismutase; TSC1/2, tuberous sclerosis complex 1 and 2; TST, tail suspension test; UCMS, unpredictable chronic mild stress.

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Conflicts of interest

There are no conflicts of interest.

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