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Pioglitazone, a PPARγ agonist rescues depression associated with obesity using chronic unpredictable mild stress model in experimental mice

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ABSTRACT

Pioglitazone, a peroxisome proliferator activated receptor gamma (PPARγ) agonist belonging to thiazolidinedione class, is mainly used in diabetes mellitus. Obese subjects are twice likely to become depressed than non-obese individuals. The biological mechanisms linking depression with obesity still remain poorly understood and there is immense need for better therapeutic intervention against such co-morbid disorders. The present study investigates the effect of pioglitazone on the chronic unpredictable mild stress (CUMS) induced depression in obese mice by using behavioral tests and biochemical estimations. Mice were fed with high fat diet (HFD) for 14 weeks and were further subjected to different stress procedures for 28 days to induce depressive behavior. Animals were administered orally with pioglitazone (30 mg/kg p.o.)/escitalopram (10 mg/kg p.o.)/vehicle (10 ml/kg p.o.) daily from day 15–28. Various behavioral paradigms such as sucrose preference test, forced swim test (FST), tail suspension test (TST) and elevated plus maze (EPM) were performed. Biochemical estimations including plasma glucose, total cholesterol, triglycerides, and total proteins were performed. The data obtained from behavioral assays and biochemical assessments indicated that obese animals exhibited severe depressive-like behavior compared to non-obese animals. Furthermore, obese animals subjected to CUMS worsen the depressive behavior compared to obese control animals. Repetitive treatment with pioglitazone reversed the CUMS induced behavioral and biochemical alterations in HFD fed obese mice which atleast in part may be mediated through improving altered plasma glucose. The study suggests that pioglitazone needs further attention with respect to molecular mechanisms that could provide a better therapeutic strategy against depression associated with obesity.

1. Introduction

National Comorbidity Survey reveals that major depressive disorder (MDD) is a debilitating disease with a prevalence rate of 16.2% (Kessler et al., 2003). In depression remission is achieved by only one third of the patients after treatment with antidepressant agents (Rush et al., 2006). Another major disease as a global burden is obesity as it is directly associated with increased morbidity from cardiovascular disease, type 2 diabetes and some cancers. Epidemiological data suggest that obesity is linked to an increased risk of depressive and mood disorders (Simon et al., 2006). The current antidepressants like citalopram and fluoxetine have been reported to show resistance in depression associated with obesity (Isingrini et al., 2010). Despite this information, there is presently little information on how the development of obesity heightens the risk for depression.

Chronic unpredictable mild stress (CUMS) is the most important pathogenic factor in several neuropsychiatric diseases such as depressive disorder, as stress exposure modifies the onset and evolution of some neurological diseases (Garcia-Bueno et al., 2008). In rodents, CUMS model is mostly used for assessing the pathophysiology of depression and to study the effect of various therapeutic interventions on CUMS induced depression (Willner, 2005). Furthermore, CUMS leads to various long term behavioral, neurochemical, neuroimmune and neuroendocrine alterations that resemble to those observed in patients with depression (Cryan and Holmes, 2005).

Clinical reports suggest that obesity and other metabolic disorders are frequently observed among the individuals seeking treatment for mood disorders (McElroy et al., 2004). Increased visceral

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fat mass heightens the risk for depression. The biological mechanisms associated with increased cardiometabolic risk may contribute to the development of mood disorders such as depression (Vogelzangs et al., 2010). The patients with metabolic syndrome or insulin resistance syndrome experience a significantly elevated risk of developing depression (Almeida et al., 2009).

Pioglitazone (PGZ),a well established drug known as peroxisome proliferator-activated receptor gamma (PPAR-γ) agonist belonging to thiozolidinodienes (TZDs) class regulates lipid metabolism, exerts potent central and peripheral anti-neuroinflammatory action and possesses neuroprotective effect (Wozniak et al., 1993; Zhao et al., 2006; Garcia-Bueno et al., 2010; Heneka and Landreth, 2011). Several clinical and pre-clinical studies reported TZDs as superior treatments for neurological and psychiatric conditions including autism (Boris et al., 2007), Alzheimer's disease (Miller et al., 2011), multiple sclerosis (Kaiser et al., 2009) and MDD (Kemp et al., 2012). Insulin resistance and impaired glucose tolerance has been observed at higher frequency in depression (Almeida et al., 2009). A bidirectional relation between mood disorders and metabolic disturbances is well evident from the literature (Barry et al., 2009). Rosgan et al. (2002) documented that treatment of insulin resistance improves depressive symptoms.

Pioglitazone is well known drug in the treatment of insulin resistance or altered plasma glucose. Considering the "insulin resistance or altered plasma glucose" as important pathogenic link for depression associated with obesity, the present study was designed to investigate the effect of pioglitazone on CUMS induced depression in obese mice using behavioral tests and biochemical estimations.

2. Methods

2.1. Experimental animals

Behavioral experiments were conducted using male Swiss albino mice (20–25 g) that were procured from Hissar Agricultural University, Hissar, India (Reg. No. 417/01/a/CPCSEA). The animals were housed under standard laboratory conditions (temperature 22 ± 2 °C and room humidity 60 ± 10%) and maintained on 12:12 h light/dark cycle and had free access to food and water. In India, Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) is established under "Prevention of Cruelty to Animals Act 1960". CPCSEA has a representative body at institute level named as Institutional Animal Ethics Committee (IAEC). The experimental procedures performed on animals were in compliance with the protocol approved by IAEC of Birla Institute of Technology & Science, Pilani, India (Protocol No. IAEC/RES/18/09).

2.2. Schedule for drug administration and behavioral tests

Animals were fed with high fat diet (HFD) for 14 weeks, prepared according to Srinivasan et al. (2005). Pioglitazone and escitalopram was obtained from Aarti Drugs Limited (Tarapur, India) and Ranbaxy Laboratories Limited (Gurgaon, India) respectively, as a generous gift sample. The diagnostic kits for estimation of plasma glucose, total cholesterol, triglycerides and total proteins were purchased from Spinreact, Girona, Spain. Pioglitazone was prepared as a suspension in 0.25% sodium carboxyl methyl cellulose (CMC) freshly every day. Pioglitazone was administered by oral gavage (p.o.) daily from day 14–28 of the CUMS procedure (Table 1). The dose of pioglitazone (30 mg/kg p.o.) was selected according to the earlier studies (Kashani et al., 2013; Sato et al., 2011; Kubota et al., 2006).

2.3. Experimental design

Sixty mice were randomized based on body weight and divided into ten different groups (n = 6/group). Group I consisted of Normal pellet diet (NPD) mice receiving vehicle by gavage (10 ml/kg p.o.), group II comprised of NPD + pioglitazone (30 mg/kg p.o.), group III comprised of NPD + CUMS control, group IV consisted of NPD + CUMS + pioglitazone (30 mg/kg p.o.), group V consisted of NPD + CUMS + escitalopram (10 mg/kg p.o.), group VI comprised of HFD control, group VII consisted of HFD + pioglitazone (30 mg/kg p.o.), group VIII comprised of HFD + CUMS control, group IX consisted of HFD + CUMS + pioglitazone (30 mg/kg p.o.) and group X comprised of HFD + CUMS + escitalopram (10 mg/kg p.o.). Initially, for one week period, animals were only subjected to different stress procedures. From day 8th to 28th along with stress, animals of group II, IV, VII and IX received pioglitazone (30 mg/kg p.o.), group V and X received escitalopram (10 mg/kg p.o.) using oral gavage daily once, whereas, group I, III, VI and VIII were administered with vehicle orally through oral gavage as a suspension of 0.25% sodium carboxyl methyl cellulose (CMC) (Table 1).

2.4. Chronic unpredictable mild stress procedure

The CUMS was performed as described earlier (Ducottet et al., 2003). Briefly, the CUMS protocol consisted of the sequential application of a variety of mild stressors. These stressors were randomly scheduled over one week period as shown in Table 2, and repeated throughout the 4 week experiment. Non-stressed animals were left undisturbed in their home cages except during housekeeping procedures such as cage cleaning.

2.5. Behavioral tests battery

2.5.1. Sucrose preference test

The test was performed as described earlier (Casarotto and Andreatini, 2007) with minor modifications. Briefly, before the test, mice were trained to adapt to sucrose solution (1%, w/v), two bottles of sucrose solution were placed in each cage for 24 h and then one bottle of sucrose solution was replaced with water for 24 h. After the adaptation, mice were deprived of water and food for 24 h. Sucrose preference test was conducted at 9:30 a.m. in which mice were housed in individual cages and were free to access to two bottles containing 100 ml of sucrose solution (1% w/v) and 100 ml of water, respectively. After 24 h, the volumes of consumed sucrose solution and water were recorded and the sucrose preference was

Table 1
Schematic representation of study protocol.

Days	0–14th day	15–28th day	29–37th day					39th day	40th day onwards
Study protocol	Chronic unpredictable mild stress (CUMS)	PGZ (30 mg/kg p.o.)/ESC (10 mg/kg p.o.)/vehicle (10 ml/kg p.o.)	Behavioral assays on day 29–33 Sucrose preference test	34 Locomotor score	35 FST	36 TST	37 EPM	Collection of blood Plasma glucose, plasma total cholesterol, plasma triglycerides, plasma total proteins	Biochemical assessment

Table 2
Schedule for chronic unpredictable mild stress (CUMS) procedure.

Sr. No.	Day	Type of stress	Time
1.	Monday	Food and water deprivation	24 h (9 a.m.–9 a.m.)
2.	Tuesday	Empty bottle	1 h (9 a.m.–10 a.m.)
		Foreign object (eg. marbles)	24 h (9 a.m.–9 a.m.)
3.	Wednesday	Overnight illumination	10 h (8 p.m.–6 a.m.)
		Forced Swimming	1 h (4 min/animal) (9 a.m.–10 a.m.)
4.	Thursday	Cage tilt	5 h (12 p.m.–5 p.m.)
		Restraint	1 h (10 a.m.–11 a.m.)
5.	Friday	Food deprivation	12 h (9 a.m.–9 p.m.)
		Soiled cage (with water)	12 h (9 p.m.–9 a.m.)
6.	Saturday	Water deprivation	12 h (10 a.m.–10 p.m.)
		Overnight illumination	12 h (7 p.m.–7 a.m.)
7.	Sunday	Empty bottles	2 h (10 a.m.–12 a.m.)
		Cage tilt	5 h (12 a.m.–5 p.m.)

calculated by the formula as described in following equation.

% Sucrose preference = [Sucrose consumption (ml)/
Water + Sucrose consumption (ml)] × 100

2.5.2. Spontaneous locomotor activity test

The spontaneous locomotor activity of HFD obese mice subjected to CUMS was measured by using actophotometer (Boissier and Simon, 1965; Engeland et al., 2003) (INCO, India) which consisted of a square arena (30 × 30 cm) and walls along with photocells just above the level of floor. On the day of experiment, before beginning of the tests, photocells were checked properly. As the beam of light is cut by the movement of animal the reading is automatically recorded by counter and displayed on the screen. Mice from all the respective groups were gently placed in the center of the box individually. After initial 1 min of acclimatization period, the locomotor activity score was recorded digitally for next 4 min in a dimly lit room. After each test, the floor was cleaned thoroughly with 75% alcohol solution to eliminate possible bias due to odors left by previous mice.

2.5.3. Forced swim test (FST)

FST was performed as described previously (Porsolt et al., 1977) with slight modification. Briefly, the mice were individually forced to swim in a 25 × 12 × 25 cm (L × B × H) filled with water (23 ± 2 °C) up to a height of 15 cm. Animals were allowed to swim for 15 min as training period 24 h before commencement of the test. On the test day, after the initial 2 min of vigorous activity, mice were observed for immobility for next 4 min. An animal was considered to be immobile whenever it remained floating passively in the water in a slightly hunched but upright position, its nose above the water surface. This immobile posture reflects a state of behavioral despair or helplessness (Santiago et al., 2010).

2.5.4. Tail suspension test (TST)

The TST is another well established animal model for screening antidepressants. The method was adopted from previous studies (Steru et al., 1985; Yamawaki et al., 2012). Briefly, mice were suspended by the bands and hung from a hook mounted 50 cm above the floor for 6 min. The time that the mice spent immobile during the 6 min of the testing period was measured. Immobility was considered as lack of all movement except for whisker movement and respiration.

2.5.5. Elevated plus maze (EPM)

The elevated plus maze was performed by the method mentioned earlier (Adeyemi et al., 2006). Briefly, it consisted of two open and two closed arms (all arms: 20 × 4 × 12 cm) made of wooden blocks elevated at a height of 25 cm from floor, which was lighten with 60 W bulb through a height of 100 cm. Each mouse was placed in the central square (5 cm × 5 cm) facing an open arm and allowed to explore the maze for 5 min of test period. The parameter measured was time spent in open arm and number of entries in open arm. The maze was cleaned with dilute alcohol in between two test sessions to get rid of residual odor.

2.6. Biochemical parameters

2.6.1. Estimation of plasma glucose, total cholesterol, triglycerides and total proteins

Two days post behavioral tests, animals were bleeded by sinus retro-orbital route for collection of plasma in a tube containing 10 μl of heparin solution and centrifuged at 10,000 rpm for 15 min. Estimation of plasma glucose (Trinder, 1969), total cholesterol (Meittini et al., 1978), triglycerides (Buccolo and David, 1973) and total proteins (Koller, 1984) was done by using commercially available kits (Spineract).

2.7. Statistical analysis

Data were analyzed using Graph Pad PRISM software version 2.01 (GraphPad Software, La Jolla, USA). One specific group of mice was assigned to one specific drug treatment condition. All the values are expressed as mean ± standard error of the mean (S.E.M.). The significance of differences between groups for behavioral and biochemical assays were analyzed using two-way analysis of variance (ANOVA) followed by *post hoc* Bonferroni test. For statistical analysis $p < 0.05$ was considered statistically significant.

3. Results

3.1. Behavioral assessments

3.1.1. Effect of pioglitazone treatment on body weight of HFD obese mice subjected to CUMS

The effect of pioglitazone on body weight is shown Fig. 1. HFD control group showed significantly ($p < 0.01$) higher body weight compared to NPD control animals. HFD mice exposed to CUMS showed significant weight reduction compared to HFD control group ($p < 0.01$). Repeated treatment with pioglitazone (30 mg/kg p.o.) and standard reference drug escitalopram (10 mg/kg p.o.) significantly [$F(9, 50) = 708.7$, $p < 0.01$] reversed the reduced weight in obese mice subjected to CUMS.

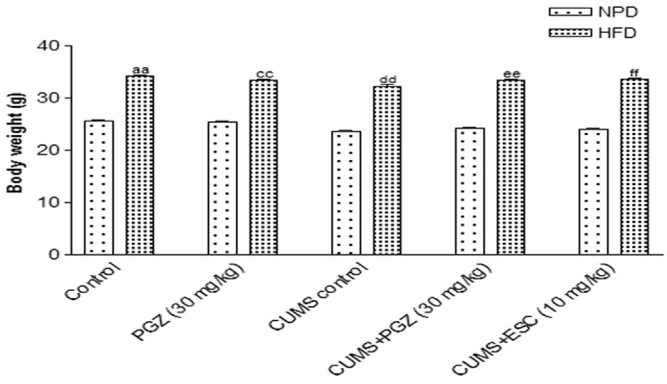

Fig. 1. Effect of PGZ (30 mg/kg p.o.) treatment on the body weight of obese mice subjected to CUMS. All the values are expressed as mean ± S.E.M., $^{aa}p < 0.01$ as compared to NPD control group, $^{cc}p < 0.01$ as compared to NPD group treated with PGZ, $^{dd}p < 0.01$ as compared to NPD + CUMS group, $^{ee}p < 0.01$ as compared to NPD + CUMS mice treated with PGZ (30 mg/kg p.o.), $^{ff}P < 0.01$ as compared to NPD + CUMS mice treated with ESC (10 mg/kg p.o.), n = 6/group.

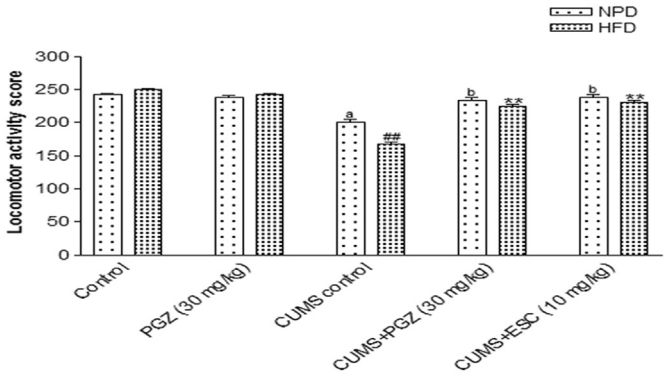

Fig. 3. Effect of PGZ (30 mg/kg p.o.) treatment on the locomotor activity score in obese mice subjected to CUMS. All the values are expressed as mean ± S.E.M., $^{aa}p < 0.01$ as compared to NPD control group, $^{b}p < 0.05$ as compared to NPD + CUMS group, $^{\#\#}p < 0.01$ as compared to HFD control group, $^{**}p < 0.01$ as compared to HFD + CUMS group, n = 6/group.

3.1.2. Effect of pioglitazone treatment on the percentage of sucrose preference in HFD obese mice subjected to CUMS

HFD control animals showed significantly ($p < 0.01$) reduced sucrose consumption compared to normal control animals (Fig. 2). HFD + CUMS control group exhibited significantly ($p < 0.01$) decreased sucrose consumption as compared to HFD control animals. Chronic treatment with pioglitazone (30 mg/kg p.o.) and standard escitalopram (10 mg/kg p.o.) showed significantly [$F(9, 50) = 14.18$, $p < 0.01$] increased sucrose consumption in obese mice subjected to CUMS.

3.1.3. Effect of pioglitazone treatment on spontaneous locomotor activity in HFD obese mice subjected to CUMS

The locomotor activity in normal, HFD obese and HFD + CUMS animals is shown in Fig. 3. No alteration was observed in the locomotor activity between the normal control and HFD control mice. However, obese mice subjected to CUMS showed significantly ($p < 0.01$) decreased locomotor activity as compared to HFD control group. Chronic treatment with pioglitazone (30 mg/kg p.o.) and standard drug escitalopram (10 mg/kg p.o.) significantly [$F(9, 50) = 9.35$, $p < 0.01$] reversed the CUMS induced reduced locomotor activity in obese mice.

3.1.4. Effect of pioglitazone treatment on immobility time in FST in HFD obese mice subjected to CUMS

Fig. 4 shows the effect of repetitive treatment on immobility time in FST in stressed obese mice. HFD control animals exhibited significantly ($p < 0.05$) increased immobility time in FST compared to normal control animals. CUMS significantly ($p < 0.05$) increased the immobility time in obese in FST compared to obese control group. Pioglitazone (30 mg/kg p.o.) and standard escitalopram (10 mg/kg p.o.) significantly [$F(9, 50) = 18.56$, $p < 0.01$] reduced the immobility time in obese mice subjected to CUMS in FST.

3.1.5. Effect of pioglitazone treatment on immobility time in TST in HFD obese mice subjected to CUMS

The effect of pioglitazone treatment on immobility time in TST is shown in Fig. 5. HFD control animals exhibited significant ($p < 0.05$) higher immobility time compared to normal control group in TST. In obese animals CUMS significantly ($p < 0.05$) increased duration of immobility as compared to HFD control group. Chronic treatment with pioglitazone (30 mg/kg p.o.) and standard reference drug escitalopram (10 mg/kg p.o.) significantly [$F(9, 50) = 9.77$, $p < 0.01$] reduced the immobility time in obese animals subjected to CUMS compared to HFD + CUMS control group in TST.

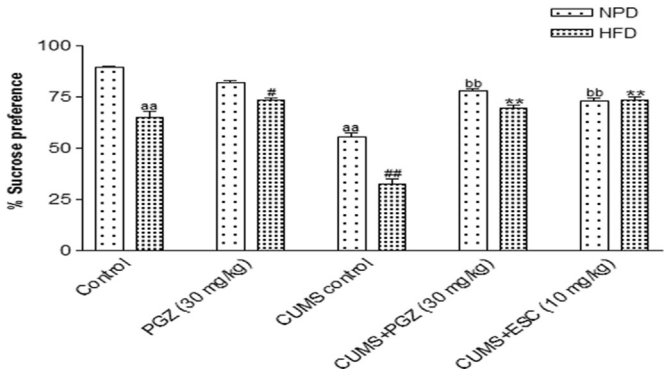

Fig. 2. Effect of PGZ (30 mg/kg p.o.) treatment on the percent sucrose preference in HFD + CUMS mice. All the values are expressed as mean ± S.E.M., $^{aa}p < 0.01$ as compared to NPD control group, $^{bb}p < 0.01$ as compared to NPD + CUMS group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ as compared to HFD control group, $^{**}p < 0.01$ as compared to HFD + CUMS group, n = 6/group.

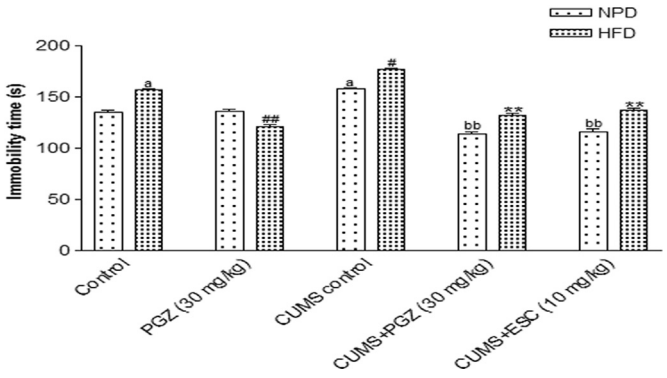

Fig. 4. Effect of PGZ (30 mg/kg p.o.) treatment on the immobility time in FST in obese mice subjected to CUMS. All the values are expressed as mean ± S.E.M., $^{a}p < 0.05$ as compared to NPD control group, $^{bb}p < 0.01$ as compared to NPD + CUMS group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ as compared to HFD control group, $^{**}p < 0.01$ as compared to HFD + CUMS group, n = 6/group.

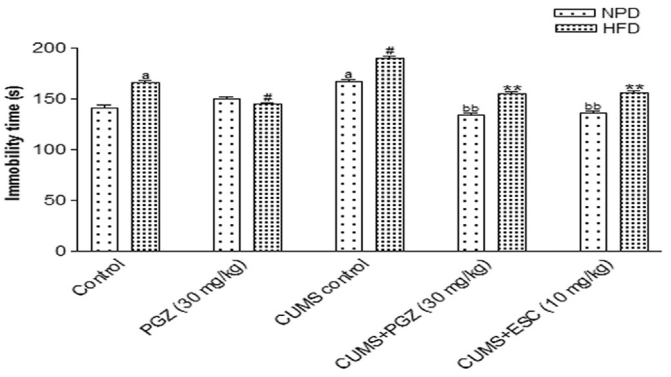

Fig. 5. Effect of PGZ (30 mg/kg p.o.) treatment on the immobility time in TST in obese mice subjected to CUMS. All the values are expressed as mean ± S.E.M., $^{a}p < 0.05$ as compared to NPD control group, $^{bb}p < 0.01$ as compared to NPD + CUMS group, $^{\#}p < 0.05$ as compared to HFD control group, $^{**}p < 0.01$ as compared to HFD + CUMS group, n = 6/group.

3.1.6. *Effect of pioglitazone treatment on EPM in HFD obese mice subjected to CUMS*

The effect of pioglitazone treatment on EPM is represented in Table 3. HFD group showed significantly ($p < 0.01$) reduced percent time spent in open arm as compared to normal control animals. HFD + CUMS animals showed significantly ($p < 0.01$) decreased time in open arm as compared to HFD control animals. However, percent open arm entries were not significantly altered in HFD and HFD + CUMS animals compared to normal and HFD control groups, respectively with pioglitazone treatment. Repetitive treatment with pioglitazone (30 mg/kg p.o.) and standard escitalopram (10 mg/kg p.o.) showed significant [$F(9, 50) = 13.11$, $p < 0.01$] increased percent time in obese animals subjected to CUMS as compared to HFD + CUMS control group. Pioglitazone (30 mg/kg p.o.) and standard escitalopram (10 mg/kg p.o.) did not showed significant effect on the percent open arm entries in obese mice subjected to CUMS.

3.2. *Biochemical analysis*

3.2.1. *Effect of pioglitazone treatment on plasma glucose, total cholesterol, triglycerides and total proteins in HFD obese mice subjected to CUMS*

The effect of pioglitazone treatment on plasma biochemical parameters is shown in Table 4. HFD control animals showed significant ($p < 0.01$) increased levels of plasma glucose, total cholesterol, triglycerides and total proteins as compared to normal control animals. Obese mice subjected to CUMS showed significant ($p < 0.05$) increased plasma glucose as compared to HFD control animals, whereas no significant alterations in plasma total cholesterol, triglycerides and total proteins were observed in obese mice subjected to CUMS compared to HFD control group. Chronic treatment with pioglitazone (30 mg/kg p.o.) and standard drug escitalopram (10 mg/kg p.o.) significantly reduced the elevated plasma glucose [$F(9, 50) = 9.89$, $p < 0.01$], total cholesterol [$F(9, 50) = 24.14$, $p < 0.05$], triglycerides [$F(9, 50) = 19.42$, $p < 0.05$] and total proteins [$F(9, 50) = 5.46$, $p < 0.01$] as compared to HFD and HFD + CUMS groups, respectively.

4. Discussion

Several meta-analysis studies demonstrated that obesity is associated with increased risk of developing depression (Zhao et al., 2009). The chronic consumption of HFD risks the anxiety and depressive-like behavior, heightens the HPA axis response to stress and leads to several biochemical modifications (Sharma and Fulton, 2012). The use of animal model for human mental disorder, despite of their oblivious limitation have proved to be of great value in the pre-clinical analysis for experimental validation of psychopharmacological assessment. It is reported that, chronic stress plays an important role in the onset and relapse of depression (Lee et al., 2002). CUMS induced depression is probably the most popular and suitable model to study depressive behavior in rodents as it possesses higher face, construct and predictive validities, reflecting the similarities in the pathogenic and behavioral alteration in human and animal depression. CUMS model aims to simulate severe depressive-like condition that is developed gradually as those are generally observed in depression patients (Luo et al., 2008). However, CUMS model provide insight but obviously cannot recapitulate the complex pathophysiology of major depressive disorder (Willner et al., 1992). Stress leads to increases triglycerides and free fatty acids. These abnormal lipid levels inhibit the release of insulin in response to glucose that further worsens the insulin resistance (Borchard, 2001). Stress also plays a crucial role in the development of systemic inflammation that are considered as metabolic syndrome by elevating the levels of pro-inflammatory cytokines such as tumor necrosis factor-alpha (TNF-α), interlukin-6 (IL-6) (Wisse, 2004). These inflammatory markers are proportional to the degree of obesity and insulin resistance. Evidence supports the role of these inflammatory markers in development of diabetes (Pradhan et al., 2001). Earlier research has made a clear indication of the relationship between stress response, visceral fat, insulin resistance and HPA axis dysregulation (Rosmond et al., 1998). It has been reported that psychosocial stress in primates leads to development of several abnormal conditions such as elevated corticosterone, insulin resistance, dyslipidemia, hypertension and coronary atherosclerosis (Jayo et al., 1993). In humans stress causes abnormal cortisol secretion that further causes metabolic disorder such as insulin resistance, diabetes and psychological diseases such as depression, anxiety (Rosmond and Bjorntorp, 2000; Raikkonen et al., 1994).

PPARγ receptors are present in various regions of the brain viz. hippocampus, striatum, frontal cortex and hypothalamus (Drew et al., 2006) and expressed on immune cells (Heneka et al., 2007). Several clinical (Kemp et al., 2006, 2012) and pre-clinical studies (Eissa et al., 2009; Sadaghiani et al., 2011) have described the antidepressant-like effects of PPARγ agonists rosiglitazone and pioglitazone.

Anhedonia indicates lack of pleasure or interest which is one of the major hallmark of human depressive symptoms and in animals this is evidenced through reduced preference for sucrose (Willner et al., 1987; Strekalova et al., 2006). Further, high predictive validity models (Cryan and Slattery, 2007; Cryan et al., 2005) like FST and TST were performed. In order to avoid the false positive results, locomotor activity score was measured and no alterations were observed in the basal locomotor activity between normal animals and HFD control animals. EPM is widely used for screening anti-anxiety agents (Hogg, 1996) where it reflects the psychomotor and emotional aspects in rodents which correlate with unconditioned anxiety. Anxiolytics elevates the frequency of entries and time spent in open arms in EPM (Dawson and Tricklebank, 1995). Overall, the result of behavioral studies examines the antidepressive-like effect of pioglitazone by improving sucrose consumption, reducing immobility time in FST and TST, increasing percent open arm time in EPM on CUMS induced depression in obese mice.

Furthermore, in the biochemical assessments, plasma glucose, total cholesterol, triglycerides and total proteins were estimated in obese mice subjected to CUMS. Plasma glucose is one of the most important biochemical assessments as “insulin resistance” is

Table 3
Effect of PGZ treatment on elevated plus maze test in HFD obese mice subjected to CUMS.

Groups	% Open arm entries	% Time in open arm (s)
NPD control	42.83 ± 4.28	19.78 ± 1.26
NPD + PGZ (30 mg/kg p.o.)	41.94 ± 3.74	18.61 ± 0.78
NPD + CUMS control	23.86 ± 3.37^{aa}	10.94 ± 1.32^{aa}
NPD + CUMS + PGZ (30 mg/kg p.o.)	48.59 ± 3.08^{bb}	18.28 ± 0.72^{bb}
NPD + CUMS + ESC (10 mg/kg p.o.)	45.25 ± 3.30^{bb}	19.06 ± 1.66^{bb}
HFD control	32.65 ± 4.40	13.39 ± 0.72^{aa}
HFD + PGZ (30 mg/kg p.o.)	38.91 ± 5.24	17.11 ± 1.07$^{\#\#}$
HFD + CUMS control	27.67 ± 3.35	6.78 ± 0.92$^{\#\#}$
HFD + CUMS + PGZ (30 mg/kg p.o.)	32.61 ± 3.26	13.89 ± 1.56**
HFD + CUMS + ESC (10 mg/kg p.o.)	29.78 ± 2.16	13.44 ± 1.09**

All the values are expressed as mean ± S.E.M., $^{aa}p < 0.01$ as compared to NPD control group, $^{bb}p < 0.01$ as compared to NPD + CUMS control, $^{\#\#}p < 0.01$ as compared to HFD control group, $^{**}p < 0.01$ as compared to HFD + CUMS control group, n = 6/group.
PGZ; pioglitazone, ESC; escitalopram, NPD; normal pellet chow, HFD; high fat diet, CUMS; chronic unpredictable mild stress.

Table 4
Effect of PGZ treatment on plasma glucose, total cholesterol, triglycerides and total proteins.

Groups	Plasma glucose (mg/dl)	Plasma total cholesterol (mg/dl)	Plasma triglycerides (mg/dl)	Plasma total proteins (g/dl)
NPD control	109.37 ± 4.39	70.09 ± 2.59	96.41 ± 5.27	18.61 ± 1.74
NPD + PGZ (30 mg/kg p.o.)	112.82 ± 4.40	69.18 ± 3.32	97.31 ± 4.27	19.75 ± 1.48
NPD + CUMS control	120.60 ± 5.91	64.43 ± 6.98	84.49 ± 4.96	22.82 ± 1.02
NPD + CUMS + PGZ (30 mg/kg p.o.)	110.79 ± 5.35	62.22 ± 5.70	73.63 ± 4.45	22.75 ± 1.43
NPD + CUMS + ESC (10 mg/kg p.o.)	105.89 ± 5.49	63.79 ± 4.52	79.56 ± 4.01	21.91 ± 2.25
HFD control	139.43 ± 8.43^{aa}	136.01 ± 5.37^{aa}	158.64 ± 5.56^{aa}	27.04 ± 2.15^{aa}
HFD + PGZ (30 mg/kg p.o.)	119.86 ± 5.10	90.14 ± 6.68$^{\#\#}$	114.60 ± 6.35$^{\#\#}$	19.17 ± 1.14$^{\#}$
HFD + CUMS control	162.45 ± 5.29$^{\#}$	116.39 ± 6.18	137.42 ± 10.42	31.87 ± 2.09
HFD + CUMS + PGZ (30 mg/kg p.o.)	131.12 ± 4.84**	94.21 ± 3.05*	111.96 ± 7.52*	23.08 ± 1.30**
HFD + CUMS + ESC (10 mg/kg p.o.)	134.47 ± 5.20**	98.70 ± 4.84	118.65 ± 4.11*	22.67 ± 1.81**

All the values are expressed as mean ± S.E.M., $^{aa}p < 0.01$ as compared to NPD control group, $^{\#}p < 0.05$; $^{\#\#}p < 0.01$ as compared to HFD control group, $^{*}p < 0.05$; $^{**}p < 0.01$ as compared to HFD + CUMS group, n = 6/group.
PGZ; pioglitazone, ESC; escitalopram, NPD; normal pellet chow, HFD; high fat diet, CUMS; chronic unpredictable mild stress.

observed in depression and obesity. Earlier reports claimed “insulin resistance” due to excess cortisol release in the circulation owing to dysregulation of hypothalamus pituitary adrenal (HPA) axis, as observed in depressed and obese subjects (Brown et al., 2004). Obesity is well known metabolic disorder in which “insulin resistance” holds the key and is characterized by dysregulation of lipids (Kahn and Flier, 2000; Lee et al., 2013). In the earlier reports it is well understood that, an association of dyslipidemia and obesity with the individuals reflecting more depressive and anxiety-like behavior (Van Reedt Dortland et al., 2010). Reduced level of total proteins in depression (Maes et al., 1995) whereas, unclear influence of total protein synthesis in obesity is well discussed previously (Anderson et al., 2008). Our study showed elevated plasma glucose, total cholesterol, triglycerides and total proteins in HFD obese mice subjected to CUMS. Pioglitazone reversed these biochemical alterations through agonistic action at PPAR-gamma receptor thereby increasing the peripheral glucose utilization. Moreover, we claim that “altered glucose or insulin resistance” as major hallmark for depressive behavior associated with obesity and treatment of the “altered glucose or insulin resistance” ameliorates these co-morbid disorders.

The results of biochemical assessments showed similarities with the earlier reports suggesting improved insulin sensitization and lipid lowering properties of pioglitazone by regulating a transcription factor responsible for glucose and fat metabolism (Srinivasan et al., 2004). From a mechanistic standpoint, improved depression severity with repetitive pioglitazone treatment may occur due to decreased in visceral adiposity and inflammation, and improved insulin sensitivity. Pioglitazone is reported to cross the blood brain barrier (Maeshiba et al., 1997) that may mediate improved depressive behavior by increasing neuronal survival (Fuenzalida et al., 2007), increasing glial uptake of excitotoxic molecules (Romera et al., 2007), or modulating Calcium dependent pathways in the brain (Pancani et al., 2009). A better understanding of the mechanisms linking insulin resistance with depression could provide therapeutic strategies with novel mechanisms. Although the exact mechanism(s) of pioglitazone is not well understood, it is known to decrease free fatty acid levels and insulin gene transcription, to remodel lipid distribution and to improve glucose disposal in insulin-resistant individuals (Schinner et al., 2009). At the molecular level, it acts as ligands for PPARγ, a nuclear receptor of the NR1C family, expressed predominantly in adipose tissue. In addition to improving glucose disposal, pioglitazone favorably altered lipid distribution in the body. Deposition of fat in the non-adipose tissue such as liver and muscle has been implicated in the development of insulin resistance. The PPARγ agonist pioglitazone is known to improve lipid distribution, insulin sensitivity and to suppress hepatic glucose production (Vikram et al., 2010). The plasma glucose and lipid profile was reduced by pioglitazone in our findings in obese animals exposed to chronic stress. This was our preliminary investigation suggesting that as pioglitazone has ability to cross BBB, future research exploring this mechanism could turn out very crucial. Also, pioglitazone has ability to improve peripheral insulin sensitivity by controlling the blood glucose and lipid distribution. Therefore, studying and exploring the mechanisms of pioglitazone including both central action and peripheral action could be very interesting area of for future research in the field of metabolic brain disorders such as co-morbid depression, anxiety associated with obesity.

5. Conclusion

In conclusion, in the present study chronic treatment with PPARγ agonist, pioglitazone reversed the CUMS induced behavioral

and biochemical changes in obese mice, thus exhibiting antidepressive-like effect. The plasma glucose level indicates that the "altered plasma glucose or insulin resistance", to play a crucial role in such co-morbid disorders and pioglitazone through activation of PPARγ receptors alleviated the "altered plasma glucose or insulin resistance" and exhibited antidepressant-like effect. However, these are only the preliminary findings and hence, further studies dealing with the role of pioglitazone with respect to molecular mechanisms including brain derived neurotrophic factor (BDNF), corticosterone levels, mRNA expression in cortex and hippocampus in depression associated with obesity will shade light on the mechanism aspects.

Conflict of interest

Authors have no conflict of interest.

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