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Research report

Chronic agmatine treatment prevents olanzapine-induced obesity and metabolic dysregulation in female rats

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ABSTRACT

Antipsychotic-induced obesity affects millions of people and is a serious health condition worldwide. Olanzapine is the most widely prescribed antipsychotic agent with high obesogenic potential. However, the exact mechanism by which it causes its metabolic dysregulation remains poorly understood. In this study, we investigated the effect of agmatine in olanzapine-induced metabolic derangements in Female Sprague-Dawley rats. Repeated olanzapine administration for 28 days increased body weight while treatment with agmatine from days 15 to 28 prevented the body weight gain induced by olanzapine without any alteration in food intake. Repeated agmatine treatment decreased the elevated feeding efficiency and adiposity index, as well as improved dysregulated lipid metabolism induced by olanzapine. Increased activity of fatty acid synthase (FAS) and decreased expression of carnitine palmitoyl transferase-1 (CPT-1) were detected in chronic olanzapine-treated rats. Although agmatine treatment did not alter FAS activity, it increased CPT-1 activity. It is possible that the inhibitory effect of agmatine on weight gain and adiposity might be associated with increased mitochondrial fatty acid oxidation and energy expenditure in olanzapine-treated rats. We suggest that agmatine can be explored for the prevention of obesity complications associated with chronic antipsychotic treatment.

1. Introduction

Obesity and associated metabolic dysregulation is a pressing priority for the global healthcare system. Particularly, obesity associated with chronic use of atypical antipsychotics is alarming and affects millions of people worldwide (Charlson et al., 2018; Hamre, 2013; Yeo and O'Rahilly, 2021). Olanzapine is the main atypical antipsychotic drug highly prescribed for the treatment of schizophrenia (Joffe et al., 2008; Lieberman et al., 2005). However, olanzapine is also one of the most obesogenic agents among second-generation antipsychotic drugs. Clinical studies have reported that nearly 86 % of patients on olanzapine therapy have shown weight gain or obesity (Kahn et al., 2008; Lieberman et al., 2005; McEvoy et al., 2007). Its long-term use causes derangements in the blood lipid and glucose profile (Himmerich et al., 2015). These metabolic adverse effects including hyperglycemia, decreased insulin sensitivity, obesity and dyslipidemia are the risk factors for cardiovascular disease leading to an increased mortality rate in schizophrenic patients (Daumit et al., 2008; Meyer et al., 2008). Moreover, chronic olanzapine treatment significantly enhanced the risk for the development of type-II diabetes in psychotic patients (Koro et al., 2002; Lambert et al., 2006). Although existing medications might offer limited relief from the metabolic dysregulation induced by antipsychotics, there is a need for effective medication which can treat the obesity induced by olanzapine.

Agmatine is an endogenous polyamine compound biosynthesized through the decarboxylation of L-arginine by arginine decarboxylase (ADC) (Reis and Regunathan, 2000). It is implicated as a potential central neuromodulator and/ or neurotransmitter in the mammalian brain, synthesized, stored in synaptic vesicles, accumulated by uptake, released by depolarization, and degraded by the enzyme agmatinase and diamine oxidase (DAO). It has a multi-receptorial affinity and acts via various neuronal pathways (Halaris and Plietz, 2007; Reis and Regunathan, 2000). Agmatine activates both I1/I2 imidazoline and

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α2-adrenergic receptors, and antagonizes N-methyl-D-aspartic acid (NMDA) receptors (Halaris and Plietz, 2007; Reis and Regunathan, 2000; Yang and Reis, 1999) and inhibits all isoforms of enzyme, nitric oxide synthase (NOS) (Auguet et al., 1995). Recently, agmatine has been explored as a therapeutic potential for obesity by decreasing hepatic lipogenesis and reducing plasma triglyceride/high-density lipoprotein cholesterol in mice (Wisniewska et al., 2021). Interestingly, it has shown a critical role in weight reduction associated with hormonal and metabolic disturbances in high-fat diet-induced obesity in rats (Nissim et al., 2014). The role of agmatine in obesity as well as metabolic complications like diabetes has been fairly established (Wu et al., 2000). Agmatine increased insulin secretion from pancreatic β-cells (Sener et al., 1989) and lowered plasma glucose in alloxan-induced diabetic rats (Jou et al., 2004). It also ameliorated insulin resistance in high-fat diet-fed rats (Su et al., 2009) and prevented the development of oxidative stress in diabetic rats (Ferents et al., 2013).

Several recent studies demonstrated the role of agmatine in fat metabolism. Agmatine is localized mainly in the cytoplasm and the vicinity of the endoplasmic reticulum and in mitochondria (Li et al., 1995; Otake et al., 1998; Reis et al., 1998). It promotes mitochondrial function by increasing cellular cAMP levels and protects against energy stress (Arndt et al., 2009; Chai et al., 2016; Cunha et al., 2016; Gardini et al., 2003; Molderings et al., 2004; Nissim et al., 2014). Given this background, we hypothesized that agmatine may prevent the obesity and metabolic dysregulation associated with chronic olanzapine treatment.

The present study was designed to understand the role of agmatine in olanzapine-induced obesity and lipid/metabolic dysregulation in female rats and its possible mechanism of action.

2. Material and methods

2.1. Subjects

Adult female Sprague–Dawley rats (200 ± 5 g body weight) after adaptation to experimental conditions were housed individually (unless indicated otherwise) in acrylic cages (24 ×17 × 12 cm). All animals were maintained at ambient room temperature (25 ± 2 °C) and relative humidity (50 ± 5 %) under 12:12 h dark-light cycle (lights on at 07:00 h). Food (Trimurti Feeds, Nagpur, India; provide 3.30 kcal/g with 23.4 % protein, 4.5 % fat, and 72.1 % carbohydrate) and water were made available ad libitum throughout the study. Experimental protocols were approved by the Institutional Animal Ethics Committee and conducted under strict compliance with the guidelines of the Committee for the Purpose of Control and Supervision of Experiments on Animals, Ministry of Environment and Forests, Government of India (CPCSEA).

2.2. Drugs and administration

Agmatine sulfate was purchased from Sigma-Aldrich Co. (Saint Louis, MO, USA). Agmatine was dissolved in saline (0.9 w/v NaCl) and preserved as stock according to storage instructions provided by the supplier. Olanzapine was dissolved in a minimum volume of glacial acetic acid (nearly 0.2 %) before the addition of saline and brought to pH 6.0 by the addition of 1 M NaOH, as suggested by Kirk et al. (2004). All drug solutions were freshly reconstituted and administered by the intraperitoneal (ip) route.

2.3. Treatment groups

Animals were divided into eight different treatment groups (n = 5–7). Group 1- Vehicle + Vehicle; Group 2- Olanzapine (1 mg/kg) + Vehicle; Group 3, 4, 5- Vehicle + Agmatine (5–20 mg/kg); Group 6–8- Olanzapine (1 mg/kg) + Agmatine (5–20 mg/kg). Schematic representation of the adopted protocol is shown in Fig. 1. Based on our preliminary studies, an acute dose of olanzapine (1 mg/kg, ip) that does not stimulate food intake or do not sedate the animals, or reduces the locomotion was selected. Moreover, this dose of olanzapine (1 mg/kg, ip) has been demonstrated to produce an antipsychotic-like effect in rodent models of schizophrenia (Ugale et al., 2004). Thus, olanzapine (1 mg/kg, ip, once daily) was administered from day 0 to day 28, and agmatine was injected (ip) 30 min before olanzapine or vehicle from day 15 to day 28 of the experiment. Olanzapine was injected daily between 09.00 and 10.00 a.m. and immediately after this, food was offered to animals.

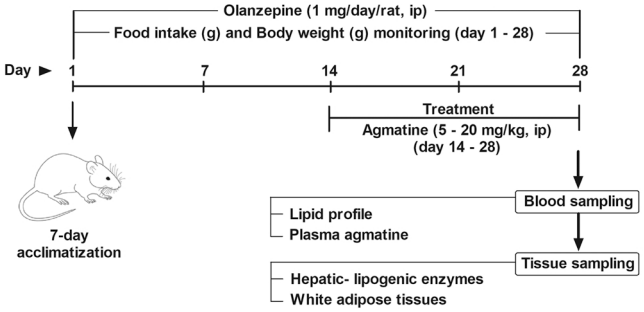

Fig. 1. The schematic outline of the experimental protocol and schedules adopted in the study. Separate groups of rats were treated with olanzapine (1 mg/kg, ip) once daily during days 0–28 and agmatine (5–20 mg kg, ip) once daily during days 15–28 of the protocol.

2.4. Measurement of food intake and body weight

Immediately after the injection of drugs, the rats were housed individually in their home cages. The pre-weighed food pellets (around 30 g) were placed into the hopper of the cage. The skilled observer blind to the treatments measured the amount of food consumed by rats, this was, however, quantified (g) by weighing manually the leftover food pellets in the hopper. The food intake and body weight of each animal was recorded daily i.e., after 24 h of the treatments (before the next injection time-point). The food spillage from the tray positioned beneath the grid floor was subtracted from the total food consumed (Kask et al., 1998; Kokare et al., 2006). In our earlier study, the spillage made by individual rats across all the groups was measured at around 0.3 g after 24 h post-injection time-point (Taksande et al., 2011). Therefore, it did not seem to affect overall feeding data. Feeding efficiency was calculated as grams of weight gain/grams of food consumed.

2.5. Biochemical estimations

2.5.1. Lipid profile

At the end of the 28th day of the experiment, animals fasted overnight and the blood was collected by the retro-orbital method. Plasma total cholesterol (TC) was determined by the cholesterol oxidase-peroxidase (CHOD-POD) method using a cholesterol assay kit (Catalog – 79960, Crystal Chem, IL, USA), and triglycerides (TG) were determined by glucose oxidase-peroxidase (GOD-POD) method using a triglyceride assay kit (Catalog – 10010303, Cayman Chemical, Michigan, USA). HDL cholesterol was determined using the earlier mentioned cholesterol assay kit (Catalog – 79970, Crystal Chem, IL, USA). LDL cholesterol was calculated using the direct endpoint method as per the manufacturer's protocol (Meiattini et al., 1978). Plasma β-hydroxybutyrate, glycerol and free fatty acid concentrations were assayed by spectrophotometry and fluorimetry using commercial test kits (Cayman Chemical Company, Michigan, USA) (Hušek et al., 2002; Li et al., 2001).

2.5.2. Estimation of plasma agmatine

From group 1 (vehicle + vehicle) and group 2 (olanzapine + vehicle) animals, blood withdrawn on day 29 was analyzed for agmatine levels by the HPLC method (Roberts et al., 2005; Zhao et al., 2002). Briefly, 100 μl plasma was transferred to a vial containing 30 μl

of trichloroacetic acid solution (30 % w/v). The solution was kept on ice for 60 min and again centrifuged at 6000 × *g* for 10 min at 4 °C (Zhao et al., 2002). The supernatant was removed and stored at − 80 °C until used for further derivatization and HPLC analysis (Roberts et al., 2005). For the pre-column derivatization of agmatine, 50 µl of prepared supernatant was transferred to a reaction vial to which 100 µl of borate buffer (pH 9.4), 40 µl of NaCN solution (0.025 M), and 100 µl of naphthalene dicarboxaldehyde (0.05 M in methanol) was added and set aside for 20 min at room temperature to produce a highly stable and fluorescent derivative of agmatine with naphthalene dicarboxaldehyde. 20 µl of this derivatized mixture was injected into a 4.6 × 250 mm cosmosil 5C8-MS HPLC cartridge and eluted with 80 % acetonitrile in a phosphate buffer (pH 6.81) at a flow rate of 1.5 ml/min. Fluorescence was recorded using an excitation wavelength of 249 nm and an emission wavelength of 466 nm. The concentration of agmatine in plasma was calculated using external standards.

2.5.3. White adipose tissue

After blood withdrawal, rats were sacrificed by the high dose of pentobarbital sodium (110 mg/kg, ip). Liver and white adipose tissues were carefully excised, rinsed with phosphate buffer saline, and weighed (Lykkegaard et al., 2008). Adiposity index was calculated as total white adipose tissue weight (g)/body weight (g) × 100.

2.5.4. Hepatic lipogenic enzymes

Metabolic impairments are usually associated with the altered functional levels of different lipogenic enzymes including fatty acid synthase (FAS) and carnitine palmitoyltransferase-1 (CPT) involved in the lipid metabolism. Excised liver homogenates were prepared and analyzed for FAS (Cloud-Clone Corporation, Houston, Texas, USA) and CPT-1 (MyBioSource, Inc., San Diego, CA, USA) activities by sandwich ELISA as per the protocol outlined by the manufacturer. Data were expressed as % relative activity of these enzymes against vehicle-treated rats.

2.6. Data analysis

The data from behavioral or biochemical experiments were subjected to statistical analysis using one- or two-way ANOVA followed by post-hoc Dunnett or Bonferroni multiple comparison tests. The data are presented as mean ± SEM and differences were considered significant at $P < 0.05$.

3. Results

3.1. Agmatine treatment prevented olanzapine-induced body weight gain

As shown in Fig. 2, repeated olanzapine (1 mg/kg, ip) administration once daily for 28 days induced significant body weight gain as compared to that of control [factor 'olanzapine treatment' $F(1, 308) = 251.38$, $P < 0.001$; factor 'duration in days' $F(27, 308) = 30.97$, $P < 0.001$ and interaction 'olanzapine treatment × duration in days' $F(27, 308) = 2.26$, $P = 0.0005$]. Chronic treatment of olanzapine in rats led to a substantial increase in body weight with a significant weight gain from day 5 onwards. Within 28 days, 23.05 % and 15.47 % weight gain of starting body weight was estimated in olanzapine (47.54 ± 2.38 g) and saline (30.27 ± 1.13 g) treated rats.

Pre-administration of agmatine (20 mg/kg, ip) prevented the body weight gain induced by olanzapine [$F_{Treatment \times Days}(81, 672) = 2.40$, $P < 0.01$; $F_{Treatment}(3, 672) = 210.75$, $P < 0.01$; $F_{Days}(27, 672) = 74.75$, $P < 0.001$]. On day 14, the average body weight gain of vehicle, olanzapine, and agmatine + olanzapine treated rats was 14.61 ± 1.33 g, 32.59 ± 5.45 g, and 31.91 ± 1.38 g respectively. Further, for 14 days (day15–28), the body weight gain recorded in the vehicle and olanzapine-treated rats were 15.66 ± 2.67 g and 14.95 ± 3.19 g respectively. Whereas, weight gain of only 1.76 ± 1.41 g was calculated from the group of rats treated with agmatine + olanzapine during the same period. Administration of agmatine for 14 days (from day 15–28) in the animals receiving saline for 28 days did not produce any significant change in the body weight gain as compared to vehicle-treated rats. Agmatine at lower doses (5 and 10 mg/kg) failed to alter the olanzapine-induced body weight gain (Supplementary data).

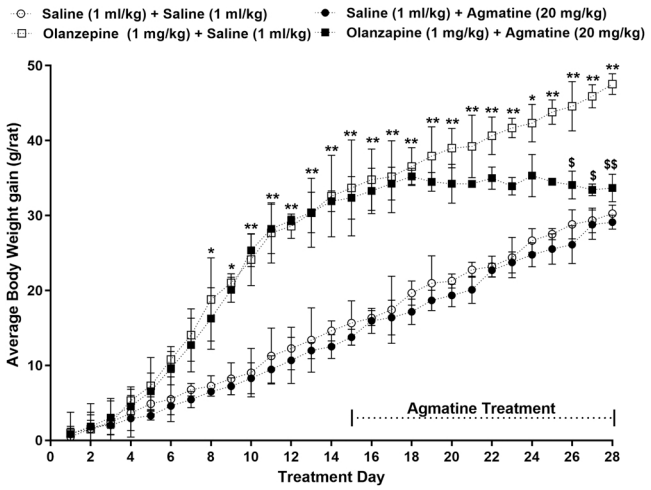

Fig. 2. Effect of agmatine treatment on chronic olanzapine-induced body weight gain in female rats. A separate group of animals received olanzapine (1 mg/kg, ip) or saline (1 ml/kg, ip) from days 1 to 28 and saline (1 ml/kg, ip) or agmatine (20 mg/kg, ip) from day 15 to 28. Each bar represents mean body weight gain (g) ± SEM (n = 6–8). *P < 0.05, **P < 0.001 vs saline + saline; #P > 0.05, ##P > 0.01 vs olanzapine + saline treated animals (Two way ANOVA post hoc Bonferroni mean comparisons).

3.2. Olanzapine and/or agmatine treatments did not affect daily food consumption

Food intake was not significantly changed by olanzapine when administered (ip) in the dose of 1 mg/kg [factor 'olanzapine treatment' $F(1, 308) = 0.54$, $P = 0.46$; factor 'duration in days' $F(27, 308) = 0.17$, $P = 1.00$ and the interaction 'olanzapine treatment × duration in days' $F(27, 308) = 0.29$, $P = 0.99$] (Fig. 3A). Similarly, no alteration in food intake was observed following the injection of agmatine (20 mg/kg, ip) to saline (1 ml/kg, ip) or olanzapine (1 mg/kg, ip) treated rats [$F_{Treatment \times Days}(81, 644) = 0.28$, $P = 1.00$; $F_{Treatment}(3, 644) = 0.33$, $P = 0.81$; $F_{Days}(27, 644) = 0.32$, $P = 0.99$] (Fig. 3A).

As shown in Fig. 3B, olanzapine (1 mg/kg) treated rats demonstrated significantly higher ($P < 0.01$) feeding efficiency ($t = 2.69$, $df = 54$, $P < 0.01$) as compared to vehicle-treated control animals. One-way ANOVA revealed that repeated agmatine treatment significantly alter elevated feeding efficiency by olanzapine (1 mg/kg) [$F(3, 111) = 2.57$, $P = 0.05$)]. Post hoc Bonferroni comparison showed significant reduction in feeding efficiency in olanzapine + agmatine (20 mg/kg) ($P < 0.05$) treated rats. However, calculated feeding efficiency in the group of animals treated with olanzapine + agmatine (5 and 10 mg/kg) was statistically insignificant when compared to olanzapine + saline-injected rats.

3.3. Effect of agmatine on lipid metabolism

Table 1 summarizes the effect of olanzapine on plasma triglycerides, total cholesterol, LDL and HDL cholesterol. A significant elevation in the plasma triglycerides by 147 % ($t = 4.39$, $df = 10$, $P < 0.01$), total cholesterol by 84 % ($t = 3.92$, $df = 10$, $P < 0.01$) and LDL-cholesterol by 216 % ($t = 5.52$, $df = 10$, $P < 0.01$) was observed in olanzapine treated animals as compared to control rats. On the other hand, there was significant reduction in the HDL-cholesterol by 58 % ($t = 5.51$,

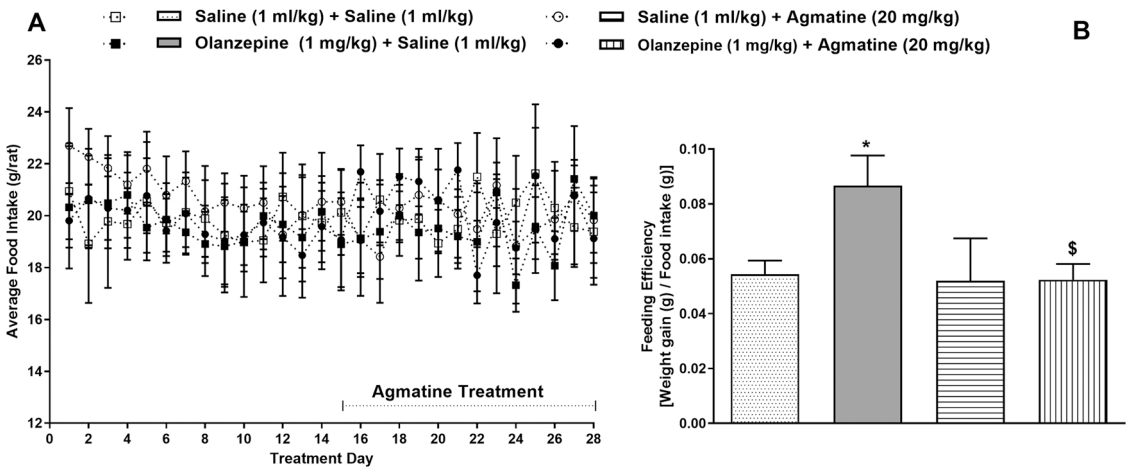

Fig. 3. Effect of agmatine on food intake (3A) and feeding efficiency (3B) in chronic olanzapine treated female rats. Separate group of animals received olanzapine (1 mg/kg, ip) (day 1–28) or saline (1 ml/kg, ip) (day 1–28) or agmatine (20 mg/kg, ip) (day 15–28) and saline (1 ml/kg, ip) (day 1–28) or olanzapine (1 mg/kg, ip) (day 1–28) and agmatine (20 mg/kg, ip) (day 15–28). Each bar represents mean food intake (g) (24 h)/feeding efficiency ± SEM (n = 6–8). *P < 0.01 vs saline + saline; $P > 0.05 vs olanzapine + saline-treated animals (One way ANOVA post hoc Bonferroni mean comparisons).

Table 1
Effect of agmatine (5–20 mg/kg, ip) on plasma lipid profile in chronic olanzapine (1 mg/kg, ip) treated female rats. Separate group of animals received olanzapine (1 mg/kg, ip) (day 1–28) or saline (1 ml/kg, ip) (day 1–28) or agmatine (20 mg/kg, ip) (day 15–28) and saline (1 ml/kg, ip) (day 1–28) or olanzapine (1 mg/kg, ip) (day 1–28) and agmatine (20 mg/kg, ip) (day 15–28). Blood samples were collected at the end of 28th day experiment with overnight fasting. Value represents mean plasma total cholesterol (mg/dl) or triglyceride (mg/dl) or LDL-cholesterol (mg/dl) or HDL-cholesterol (mg/dl) ± SEM (n = 6). $P < 0.05, $$P < 0.01 vs saline + saline; *P < 0.05 vs olanzapine + saline treated rats.

Treatment group	Total cholesterol (mg/dl)	Triglyceride (mg/dl)	LDL cholesterol (mg/dl)	HDL cholesterol (mg/dl)
Saline + Saline	97.01 ± 8.19	51.37 ± 9.12	33.17 ± 4.61	47.33 ± 2.29
Olanzapine + Saline	178.56 ± 19.13$	127.32 ± 14.67$$	124.87 ± 15.96$$	20.16 ± 4.36$
Olanzapine + Agmatine (5)	152.23 ± 8.77	109.49 ± 13.88	91.47 ± 9.85	26.69 ± 7.85
Olanzapine + Agmatine (10)	136.15 ± 17.41	81.24 ± 6.95	74.81 ± 18.72	30.48 ± 2.74
Olanzapine + Agmatine (20)	101.03 ± 9.33*	70.16 ± 1.44*	48.91 ± 7.39*	43.55 ± 1.83*

df = 10, P < 0.01). This data supports obesity induction and metabolic alteration by chronic treatment with olanzapine. Treatment with agmatine (5–20 mg/kg), from 15th day in olanzapine-receiving rats, improved the plasma markers of lipid metabolism as compared to saline-treated animals. While agmatine (20 mg/kg) injected rats significantly decreased plasma total cholesterol [F (4, 29) = 6.63, P < 0.01], triglycerides [F (4, 29) = 8.57, P < 0.01], LDL-cholesterol [F (4, 29) = 8.34, P < 0.01] levels by 43 % (P < 0.05), 44 % (P < 0.05), 60 % (P < 0.05) respectively, when compared against olanzapine-saline treated animals, significant increase in plasma HDL-cholesterol by 116 % (P < 0.01) was observed in agmatine 20 mg/kg treated rats [F (4, 29) = 6.79, P < 0.01]. Although the normalization of lipid parameters in animals receiving agmatine 5 and 10 mg/kg was observed, the alterations in the values were statistically insignificant.

3.4. Plasma agmatine levels in olanzapine-treated rats

Plasma agmatine levels in chronically (28 days) olanzapine and saline-treated rats were 9.37 ± 1.84 and 5.79 ± 2.96 ng/ml (n = 6). Statistical comparisons using 't-test suggested an insignificant decrease in plasma agmatine with olanzapine treatment as compared to control rats (t = 1.03, df = 10, P = 0.33).

3.5. Effect of agmatine on white adipose tissues weights and adiposity index in olanzapine-treated animals

As depicted in Fig. 4, significantly higher white adipose tissue mass was observed in rats following chronic olanzapine treatment for 28 days (t = 3.59, df = 10, P < 0.01). Agmatine (20 mg/kg) treated animals showed significant reduction in total white adipose tissues weight elevated by chronic olanzapine treatment [F (4, 29) = 4.18,P < 0.01] (Fig. 4A). Post hoc analysis showed a significant reduction in total white adipose tissue weights in agmatine (20 mg/kg) (P < 0.05) treated rats as compared to the olanzapine group. However, the reduction in adipose tissue weights in agmatine (5 and 10 mg/kg) treated animals was statistically insignificant.

As depicted in Fig. 4B, adiposity index was higher in the animals treated with olanzapine for 28 days (t = 2.71, df = 10, P < 0.05). Agmatine (20 mg/kg, ip) (P < 0.05) treatment from day 15–28, however demonstrated significant reduction in the adiposity [F (4, 29) = 4.05, P < 0.05]. Adiposity index in rats treated with lower doses of agmatine was not significantly different from olanzapine + saline control rats.

3.6. Effect of agmatine on the histopathology of adipose tissue of rats

The representative histological images of the adipose tissue of the different groups are shown in Fig. 5. The adipose tissue from rats in the normal control group showed normal histological features with the shape and size of adipocytes (Fig. 5A). The size of adipocytes of the olanzapine treated group was larger, showed marked enlargement of adipose cells, suggestive of hypertrophy of adipose tissue (Fig. 5B). The adipocytes of agmatine (5 and 10 mg/kg) treated do not show any marked reduction of adipocytes size as compared to the disease control group (Fig. 5C–D). The adipocytes of agmatine (20 mg/kg) showed a marked decrease in adipocytes size and eventually hypertrophy (Fig. 5E). The number of adipocytes per high power field in the disease control group were significantly reduced (p < 0.01) when compared to the normal control group owing to the hypertrophy of adipocytes. Agmatine (20 mg/kg) treated rats showed a significant increase in the

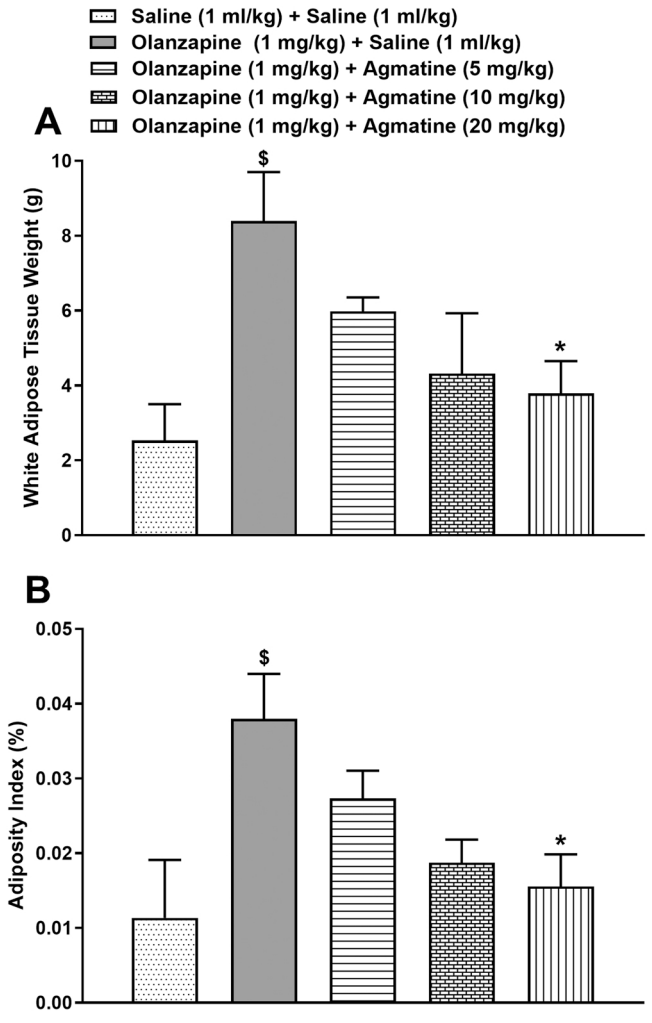

Fig. 4. Effect of agmatine (5–20 mg/kg, ip) on adipose tissue weight (g) (4A) and % adiposity index (4B) in chronic olanzapine (1 mg/kg, ip) treated rats. Each bar represents mean adipose tissue weight (g) or % adiposity index calculated as total white adipose tissue weight (g)/body weight (g) ± SEM (n = 6). $P < 0.05 vs saline + saline; *P < 0.05 vs olanzapine + saline-treated rats (One way ANOVA post hoc Bonferroni mean comparisons).

number of adipocytes per high power field when compared to the disease control group ($p < 0.001$) (Fig. 5F).

3.7. Effect of agmatine on weight of liver of rats

Table 2 summarizes the effect of agmatine (5–20 mg/kg, ip) treatment on liver weight of chronic olanzapine treated rats. A significant increase in liver weight ($t = 5.361$, $df = 4$, $P < 0.01$) was observed in chronic olanzapine treated animals as compared to control animals. Agmatine (20 mg/kg, ip), ($t = 4.757$, $df = 4$, $P < 0.001$) significantly reduced the liver weight of olanzapine treated animals.

3.8. Effect of agmatine on lipolytic biomarkers

To determine the effect of agmatine on fatty acid oxidation, we measured metabolites of lipid metabolism in plasma. Chronic olanzapine treatment for 28 days significantly increased ($t = 3.48$, $df = 10$, $P < 0.01$) plasma β-OH butyrate levels while agmatine (20 mg/kg, ip) ($P < 0.05$) treatment significantly decreased the same [$F (4, 29) = 4.38$, $P < 0.01$] (Fig. 6A). On the other hand, decreased plasma free fatty acid ($t = 4.17$, $df = 10$, $P < 0.01$) (Fig. 6B) and glycerol ($t = 7.32$, $df = 10$, $P < 0.001$) (Fig. 6C) was observed following chronic olanzapine treatment. Plasma levels of free fatty acid and glycerol are the indicators of lipolysis. Treatment of agmatine (5–20 mg/kg, ip) from day 15–28 significantly increased the rate of lipolysis as evident from the elevated plasma free fatty acid [$F (4, 29) = 4.27$, $P < 0.05$] and glycerol [$F (4, 29) = 6.87$, $P < 0.01$]. Post hoc mean comparisons indicated a significant difference in the plasma levels of free fatty acid ($P < 0.05$) and glycerol ($P < 0.01$) in the animals treated with agmatine (20 mg/kg but not 5 and 10 mg/kg) when compared against chronic olanzapine treated control animals.

3.9. Effect of agmatine on the expression of enzymes involved in fatty acid oxidation

Increased activities of FAS were detected in chronic olanzapine treated animals ($t = 4.01$, $df = 10$, $P < 0.01$). Administration of agmatine to the chronically olanzapine-treated rats did not alter the increased expression of olanzapine treatment (Fig. 7A). As shown in (Fig. 7B), the activity of CPT-1 was significantly reduced (26 %) in the animals receiving olanzapine for 28 days ($t = 3.66$, $df = 10$, $P < 0.01$) indicating the possible impairment of fatty acid oxidation. Further, agmatine (20 mg/kg, ip) ($P < 0.01$) treated rats demonstrated the significantly elevated CPT-1 activity as compared to olanzapine treated control group [$F (4, 29) = 3.87$, $P < 0.05$]. Animals that received the treatment of lower doses of agmatine (5 and 10 mg/kg) did not show any significant changes in the expression of these enzymes from olanzapine treatment.

4. Discussion

The present investigation was performed to examine the effect of agmatine on weight gain and adiposity induced by chronic olanzapine (1 mg/kg, IP) treatment. The protocols were devised with the primary objective of determining the impact of agmatine on obesity associated with chronic olanzapine therapy. After 15 days of olanzapine therapy, agmatine was administered. Importantly, previous research has shown that prolonged agmatine administration reduces weight gain and diminishes the hormonal and metabolic abnormalities associated with obesity resulting from a high-fat diet (Nissim et al., 2014). As a result, we used agmatine in combination with olanzapine for a long time (Day 15–day 28). Our findings corroborate prior research on the effect of olanzapine on weight gain in female rats (Albaugh et al., 2006; Han et al., 2008; Kao et al., 2018). However, we could not observe any hyperphagic effect at this dose of olanzapine. Thus, our findings are mostly consistent with those of Weston-Green et al. (2011), who reported significant weight gain at 0.5 and 1 mg/kg olanzapine doses without any impact on food consumption. These findings might be attributable in part to olanzapine-induced reduced energy expenditure and adipogenesis, although they may be independent of energy consumption.

Olanzapine itself is known to cause a temporary decrease in core body temperature and reduced locomotion (Evers et al., 2010; Van der Zwaal et al., 2012). The absence of hyperphagia in our study could be explained in terms of differing methodologies (e.g., diet composition, palatability, texture, etc). Alternatively, olanzapine-induced hyperphagia in rats may be ephemeral due to the drug's short duration of effect (Aravagiri et al., 1999). As a result, 24-h food consumption may not be increased. Furthermore, even when administered as a single daily injection, olanzapine consistently produced weight growth in female rats (Fell et al., 2004; Minet-Ringuet et al., 2006). It is crucial to note that the dosage of olanzapine used here has been shown to elicit an antipsychotic effect in rat models of schizophrenia and enhances feeding efficiency in rats (Patil et al., 2006; Ugale et al., 2004).

Interestingly, chronic agmatine treatment had almost no effect on food intake, but it did significantly reduce body weight gain in rats treated with olanzapine. These findings also corroborate previous evidence that agmatine reduces weight gain in rats fed with a high-fat diet (Nissim et al., 2014). Therefore, it is plausible that agmatine might trigger the molecular systems that regulate body weight homeostasis.

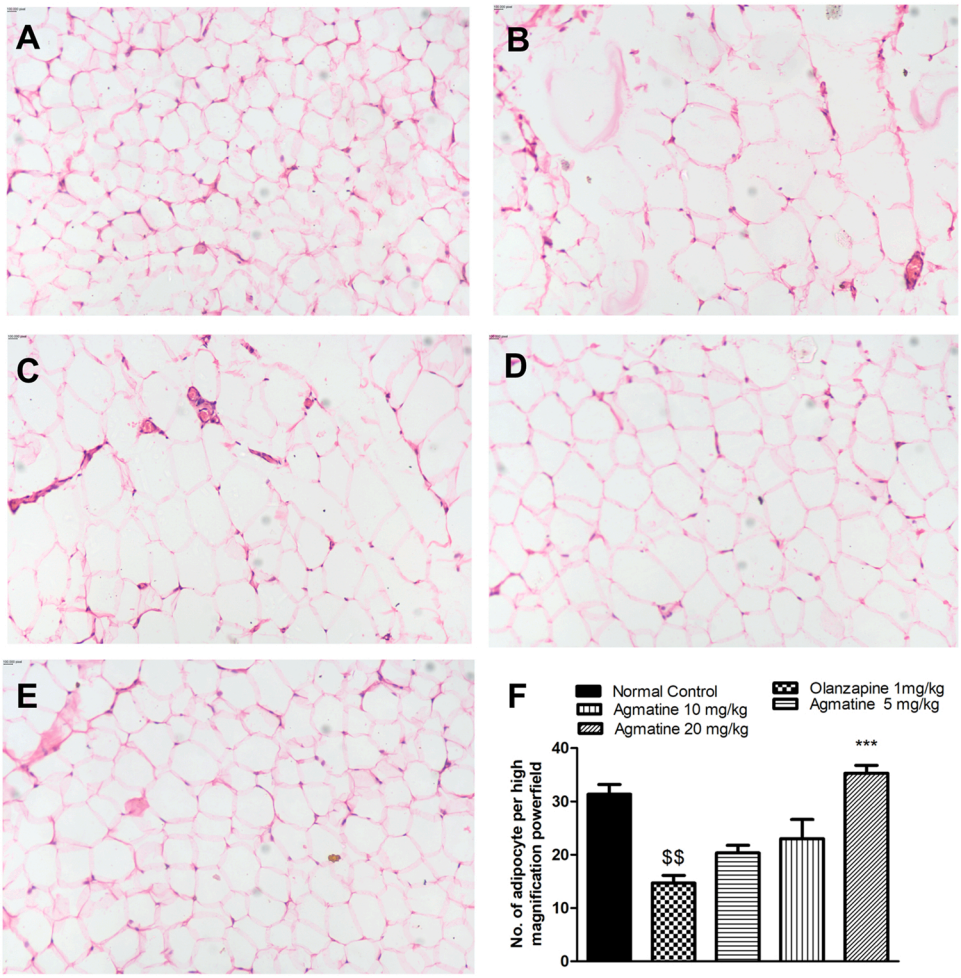

Fig. 5. Effect of Agmatine (5–20 mg/kg, ip) on the histopathology of adipose tissue in olanzapine treated rats. Adipose tissue was stained using hematoxylin and eosin, (A) Adipose tissue from rats from the control group showing normal adipocytes, (B) Adipose tissue from olanzapine treated group showing hypertrophy of adipocytes, (C and D) Adipose tissue from rats along with the agmatine 5 and 10 mg/kg respectively. (E) Adipose tissue from olanzapine-treated rats along with the agmatine 20 mg/kg showed marked decrease in hypertrophy. (F) Number of adipocytes per high power field of the different groups. (n = 3). $ $P < 0.01$ vs saline + saline; ***P < 0.01 vs olanzapine + saline treated control animals when analyzed by one way ANOVA followed by Bonferroni multiple comparison test. Magnification: 200 ×.

Table 2
Effect of agmatine (5–20 mg/kg, ip) on liver weight in chronic olanzapine (1 mg/kg, ip) treated female rats. Separate group of animals received olanzapine (1 mg/kg, ip) (day 1–28) or saline (1 ml/kg, ip) (day 1–28) or agmatine (20 mg/kg, ip) (day 15–28) and saline (1 ml/kg, ip) (day 1–28) or olanzapine (1 mg/kg, ip) (day 1–28) and agmatine (20 mg/kg, ip) (day 15–28). Blood samples were collected at the end of 28th day experiment with overnight fasting. Value represents body weights (g) and liver weight (g) of animals ± SEM (n = 6). $P < 0.001$ vs Olanzapine + Agmatine (20 mg/kg); *P < 0.01 vs Olanzapine + Saline treated rats.

Sr. no.	Treatment group	Body weight of animal (g)	Weight of liver (g)
1.	Saline + Saline	234	5.547 ± 0.452 g
2.	Olanzapine + Saline	310	9.217 ± 0.388 g *
3.	Olanzapine + Agmatine (5)	267	6.601 ± 0.216 g
4.	Olanzapine + Agmatine (10)	232	5.611 ± 0.336 g
5.	Olanzapine + Agmatine (20)	226	4.354 ± 0.318 g $

Olanzapine-induced weight gain is linked to an increase in body fat mass (Eder et al., 2001; Pouzet et al., 2003). In the current investigation, we also found a substantial elevation, in white adipose tissue weight and obesity index, in olanzapine-treated rats. The current study's robust positive connection between total white adipose tissue fat and body weight gain implies that olanzapine-induced weight gain is predominantly due to enhanced white fat deposition. Evidence suggests that olanzapine functions directly in the periphery and enhances fat deposits. For example, olanzapine induces adipogenesis in vitro through enhanced expression of sterol regulatory element-binding protein 1 (SREBP-1), which together with its related genes promotes the production of adipocytes (Yang et al., 2007). Olanzapine also promotes triglyceride accumulation and differentiation of pre-adipocytes to mature adipocytes in vitro (Yang et al., 2007). In our study olanzapine (1 mg/kg) significantly showed hypertrophy of white adipose tissue through adipocyte differentiation. Agmatine (20 mg/kg) significantly reversed the hypertrophy of adipocytes caused by chronic olanzapine treatment. Furthermore, agmatine (10 and 20 mg/kg) significantly reduced white adipose tissue weight and adiposity index in the olanzapine-treated group. Results in the current investigation imply that agmatine's ability to reduce white adipose tissue in rats may be directly connected to its potential to restrict weight gain in rats. Fatty acid synthase (FAS) is a multi-enzyme protein that catalyzes the fatty acid synthesis and is being investigated as a possible drug target for treating metabolic syndrome (Garrido et al., 2015; Kusunoki et al., 2006). The carnitine palmitoyltransferase-1 (CPT-1) regulates β-oxidation by controlling the movement of the acylcarnitine from the cytosol into mitochondria and plays important role in the genesis and treatment of metabolic disorders (Schlaepfer and Joshi, 2020). We have assessed the role of agmatine on weight of liver, there was significant increase in liver weight of chronic olanzapine treated rats which was significantly reversed by agmatine (20 mg/kg, ip).

In addition to its weight-loss effect, agmatine was able to improve some lipid metabolites in olanzapine-treated rats, including total cholesterol, triglycerides, and LDL-cholesterol levels. Obesity, particularly abdominal obesity, has been linked to dyslipidaemia, characterized

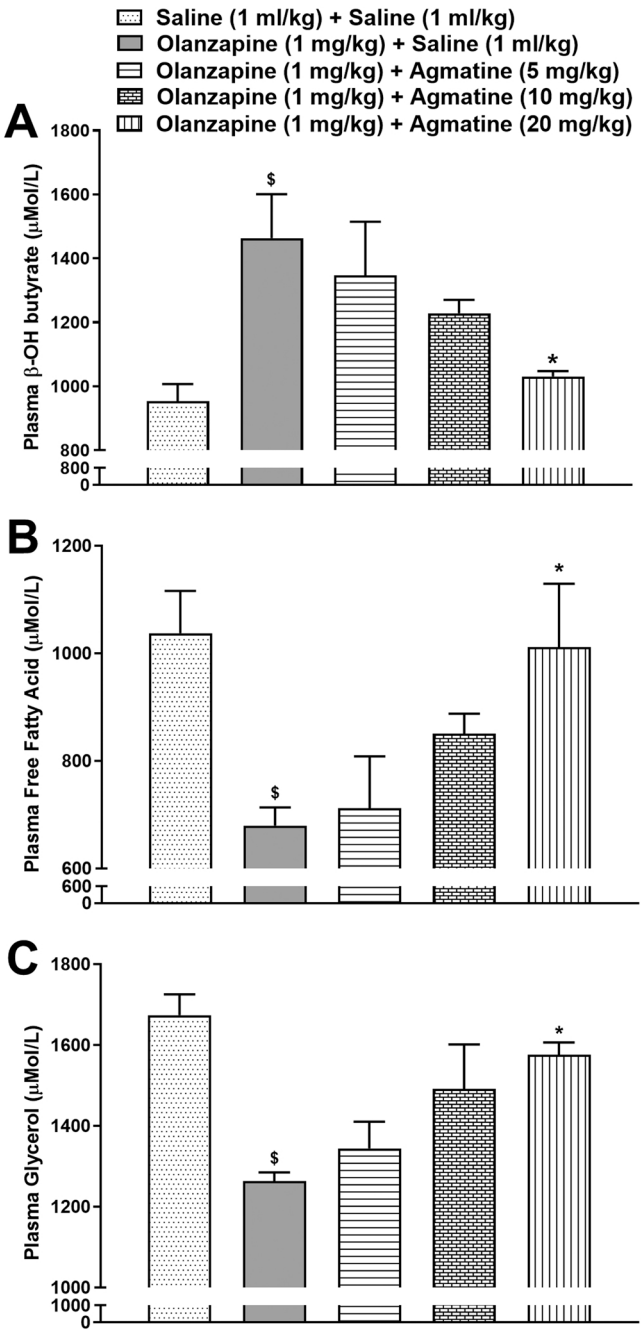

Fig. 6. Effect of agmatine (5–20 mg/kg, ip) on plasma free fatty acid (6 A & B) and glycerol (6 C) levels in chronic olanzapine (1 mg/kg, ip) treated rats. Each point represents plasma free fatty acid (μMol/L) or glycerol (μMol/L) ± SEM (n = 6). $P < 0.05 vs saline + saline; *P < 0.05 vs olanzapine + saline treated control animals.

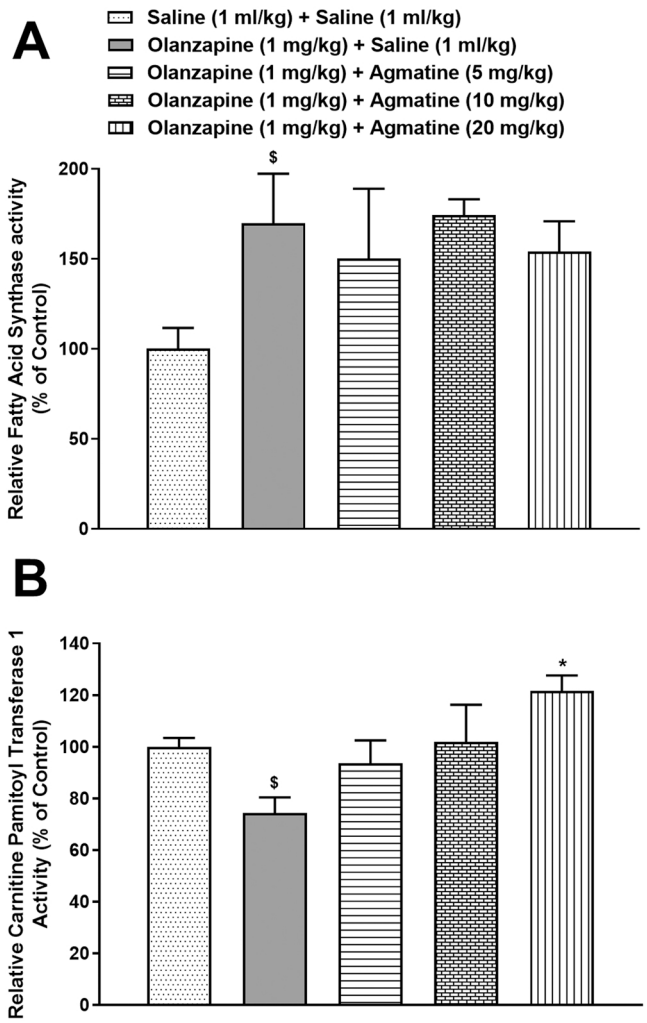

Fig. 7. Effect of agmatine (5–20 mg/kg, ip) on liver enzymes of lipid metabolism in chronic olanzapine (1 mg/kg, ip) treated rats. Each point represents relative activities (%) of liver fatty acid synthase (FAS) (7 A), and carnitine palmitoyltransferase I (CPT-1) (7B) calculated considering 100 % in vehicle treated control ± SEM (n = 6). $P < 0.05, $$P < 0.01 vs saline + saline; *P < 0.05, **P < 0.01 vs olanzapine + saline treated control animals.

by elevated triglycerides. Triglycerides play a role in the ectopic accumulation of lipid stores in the liver and are linked to a variety of diseases, including metabolic syndrome. LDL-C transports cholesterol and triglycerides to the tissues. Therefore, high total cholesterol, triglycerides, and LDL-cholesterol levels are risk factors for coronary heart disease (Xu et al., 2012). Based on these findings, we believe that agmatine could aid in the prevention of obesity complications associated with chronic antipsychotic treatment.

Substantial weight gain by olanzapine may be attributed to decreased energy expenditure and adipogenesis, and it may be independent of energy consumption. Treatment with agmatine reduced the elevated feeding efficiency and adiposity index, as well as improved the dysregulated lipid metabolism caused by chronic olanzapine treatment. Chronic olanzapine treatment in rats resulted in increased fatty acid synthase (FAS) activity and decreased expression of carnitine palmitoyl transferase-1 (CPT-1). Even though agmatine treatment did not affect FAS, it did increase CPT-1 expression, which influenced body weight. Importantly, we did not observe any mortality in agmatine or olanzapine/agmatine treated animals providing evidences for the safety profile of agmatine. Likewise, the safety of agmatine has been widely accepted and validated by several experimental and clinical studies (Bergin et al., 2019; Rosenberg et al., 2020). In olanzapine-treated rats, we speculated that agmatine's inhibitory effect on weight gain and adiposity was associated with increased mitochondrial fatty acid oxidation and energy expenditure. It is important to note that agmatine produces its pharmacological action by activating multiple receptor systems. Agmatine activates both I1/I2 imidazoline and α_2-adrenergic receptors and antagonizes blocks N-methyl-D-aspartic acid (NMDA) receptors (Halaris and Plietz, 2007; Reis and Regunathan, 2000; Yang and Reis, 1999) and inhibits all isoforms of enzyme, nitric oxide synthase (NOS) (Auguet et al., 1995). Therefore, the involvement of these receptors in the action of agmatine cannot be ruled out and needs further investigation.

In conclusion, agmatine treatment significantly decreased body

weight gain and abdominal white adipose tissue weight, and elevated obesity index induced by chronic olanzapine treatment. Furthermore, agmatine significantly decreased total cholesterol, triglycerides, and LDL cholesterol. Given that agmatine exhibits an antipsychotic-like effect in a rodent model of schizophrenia, it may offer novel therapeutic strategies for reducing olanzapine-induced body weight gain while retaining its therapeutic efficacy.

CRediT authorship contribution statement

Madhura P. Dixit: Writing – original draft, Data curation, Formal analysis, Investigation, Methodology. **Shivkumar S. Sammeta:** Methodology, Formal analysis. **Mrunali D. Dhokne:** Data curation, Methodology. **Shubhada Mangrulkar:** Resources, Methodology, Formal analysis, Writing – review & editing. **Manoj A. Upadhya:** Investigation, Methodology, Formal analysis. **Milind J. Umekar:** Resources, Methodology, Visualization, Formal analysis, Writing – review & editing. **Brijesh G. Taksande:** Resources, Visualization, Supervision, Formal analysis, Writing – review & editing. **Nandkishor Kotagale:** Conceptualization, Resources, Methodology, Formal analysis, Writing – review & editing.

Conflict of Interest

Authors declare that there is no conflict of interest/s with this manuscript.

Data Availability

Data will be made available on request.

Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at doi:10.1016/j.brainresbull.2022.10.013.

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