



Research Article

Hydroalcoholic extract of *Anethum graveolens* L. attenuates olanzapine-induced metabolic changes and locomotor activity in rats

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Abstract

Background: Olanzapine, an atypical antipsychotic, is widely associated with metabolic side effects such as excessive weight gain, dyslipidemia, and glucose intolerance. This study investigates the effect of *Anethum graveolens* L. (AG) extract on weight gain, food intake, and glucose and lipid metabolism in rats with olanzapine-induced weight gain. **Materials and Methods:** The AG extract was prepared by macerating the coarse plant powder with 70% ethanol for seven days, followed by percolation of the marc. The combined filtrates were concentrated under reduced pressure. A total of five groups were used, each comprising six animals. Three doses of the AG extract (100, 200, and 400 mg/kg, p.o.) were co-administered with olanzapine (2 mg/kg, i.p.) for 21 days. Body weight and food intake were recorded every three days, while locomotor activity was assessed weekly. After 21 days of treatment, an oral glucose tolerance test (OGTT) and lipid profile estimation were performed. **Results:** Co-treatment with the hydroalcoholic extract of AG significantly reversed olanzapine-induced weight gain and hyperphagia. Moreover, the treatment improved pancreatic β -cell function, as well as glucose and lipid metabolism. These findings suggest that AG extract mitigates the metabolic disturbances induced by olanzapine through the modulation of pancreatic function and lipid homeostasis. **Conclusion:** AG extract effectively mitigated olanzapine-induced metabolic alterations, suggesting its potential as a natural adjunct for managing antipsychotic-associated metabolic side effects.

Keywords: *Anethum graveolens* L., Lipid metabolism, Locomotor activity, Olanzapine-induced obesity, Weight gain

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Introduction

Olanzapine, one of the atypical anti-psychotic molecules, is important to manage multiple psychiatric disorders, including major depression and schizophrenia (1), over typical anti-psychotics due to their better tolerability and minimal extrapyramidal side effects. Although olanzapine possesses a better therapeutic outcome in the management of psychiatric illness, it possesses multiple metabolic disturbances, including

weight gain, mild hyperglycemia, elevated systolic blood pressure, and dyslipidemia (2-6).

Drug-repurposing approaches have been made to manage olanzapine-induced obesity by utilizing metformin, fluoxetine, orlistat, and betahistine (7-10), which are single protein-targeting agents. Another approach to manage olanzapine-induced obesity has been made by switching to another antipsychotic drug like clozapine (11); however, it is a short-term approach. Since either deregulated energy metabolism or drug-induced obesity is a polygenic condition and involves multiple proteins, to reflect the pathogenesis of obesity. Hence, identifying a new agent molecule to manage this condition is still in demand.

Multiple traditional medicines recorded by Ayurveda and other complementary medicines add to its importance in managing multiple diseases, as they compose multiple secondary metabolites to target various infectious and non-infectious diseases. *Anethum graveolens* L., commonly known as Dill,

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belongs to the family Apiaceae; reported to contain coumarins, flavonoids, phenolic acids, and steroids (12). Further, *Anethum graveolens* L. has its importance in the category of "Spice" and is consumed daily by the majority of the population in the majority of Asian countries, including Bangladesh, China, India, Nepal, and Pakistan. Further, *Anethum graveolens* L. possesses its importance as carminative, stomachic, and diuretic. Further, it is also reported for its potential in managing obesity (13) and diabetes mellitus (14). Likewise, the anti-depressant activity of *Anethum graveolens* L. (15) has also been reported. However, no reports have been found to evaluate the effect of *Anethum graveolens* L. seed extract on olanzapine-induced weight gain and lipid and glucose metabolism.

Hence, the present study investigated the effects of the hydroalcoholic extract of seeds of *Anethum graveolens* L. (HEAG) on body weight, food intake, and locomotor activity in an established female rat model of olanzapine-induced weight gain. Further, the study also investigated the effects of the extract on lipid and glucose metabolism in olanzapine-treated rats.

Materials and Methods

Collection, authentication, and extract preparation

Seeds of wild-grown *Anethum graveolens* L. were collected from local areas of Belagavi, India, washed under running water to remove foreign matter, shade dried, and turned into a coarse powder. The collected plant was authenticated at ICMR-NITM Belagavi, and the herbarium was deposited for the same for future reference (RMRC-1567). For extract preparation, the coarse powder was extracted as explained by Khanal & Patil et al (16). Briefly, the coarse powder was subjected to maceration with 70% v/v ethanol for seven days, filtered, and the marc was subjected to Soxhlet extraction; both filtrates were combined and concentrated using a rotator evaporator (IKA RV 10) under reduced pressure.

Animals and ethical clearance

Healthy SD female rats weighing 180±10 grams of either sex were purchased from CPCSEA-registered vendor and housed in pathogen-free conditions after ethical approval from Institutional Animal Ethical Committee (IAEC) at KLE College of Pharmacy, Belagavi (KLECOP/CPCSEA-Reg.No.221/Po/Re/S/2000/CPCSEA.Reg.28-12/10/2019). Animals were acclimatized under a 12 light/dark cycle for 7 days before the study.

Grouping of animals

Animals were randomized into 5 different groups containing six animals in each using computer-generated random numbers namely (a) control (CON): receives vehicle; (b) OLZ: receives olanzapine 2 mg/kg, i.p., b.i.d (5); (c) OLZ+AG100: receives olanzapine 2 mg/kg, i.p., b.i.d.+AG extract 100 mg/kg, p.o., OD; (d) OLZ+AG200: olanzapine 2 mg/kg, i.p., b.i.d.+ AG extract 200 mg/kg, p.o., OD; (e) OLZ+AG400: olanzapine 2 mg/kg, i.p., b.i.d.+AG extract 400 mg/kg. Each group contained three cages with two animals in each.

Measurement of food intake and body weight

The average food intake in each group/week was calculated using the following formula.

$$\text{average food intake (grams)} = \frac{\text{total food intake in a week}}{7}$$

Similarly, the body weight and food intake were recorded from the 1st to 21st day. The percentage weight gain was calculated using as

$$\% \text{ gain in body weigh for the corresponding day} = \frac{(\text{weight of } n\text{th day} - \text{weight of } (n - 1)\text{th day})}{\text{weight of } (n - 1)\text{th day}}$$

Locomotor activity

The animal locomotor behavior was examined using an Actophotometer. Briefly, individual animals were placed in an actophotometer for 5 min to record the basal locomotor activity of animals, followed by administration of the drug; after 30 min, locomotor activity was recorded as digital counts. The increased counts reflected the increased locomotor activity.

Oral Glucose Tolerance Test (OGTT)

OGTT was performed after 21 days of treatment as explained by Kumar et al (17). The XY plot of glucose level vs. time (min) was used to calculate the total area under the curve (AUC).

Plasma biochemical estimations

Fasting blood glucose level was measured using a glucometer (Janaushadi, India). Similarly, high-density lipoprotein (HDL), total cholesterol (TC), and triglyceride (TG) were measured using commercially available kits (ERBA diagnostics).

Statistical analysis

All data are expressed as mean ± SD. Percentage body weight gain, food intake, and oral glucose tolerance test (OGTT) data were analyzed using two-way ANOVA followed by Bonferroni's multiple comparison test. Locomotor activity was analyzed using a paired Student's t-test. The area under the curve (AUC) of glucose during OGTT and serum biochemical parameters including total cholesterol (TC), triglycerides (TG), high-density lipoprotein (HDL), and low-density lipoprotein (LDL) were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. Statistical analyses were performed using GraphPad Prism 5. Differences among group means were considered statistically significant at $p < 0.05$.

Results

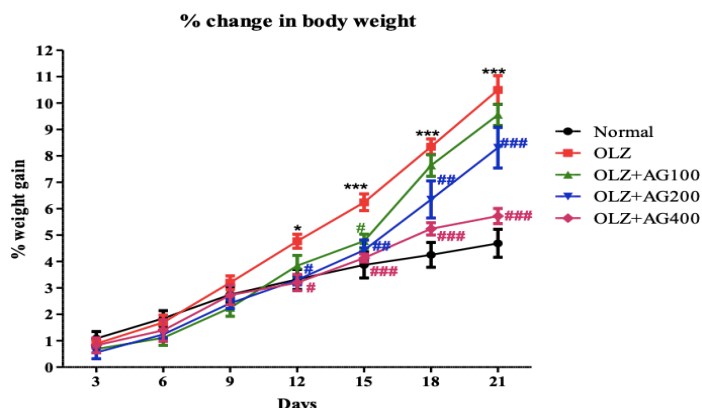
Effect on percentage body weight

There was a significant increase ($p < 0.05$ & 0.001) in percentage weight gain in the olanzapine-treated group from the 12th day to the 21st day. Further, co-treatment with HEAG at the dose of 100 mg/kg significantly reduced ($p < 0.05$) the % weight gain on the 15th day. Similarly, there was a significant decrease ($p < 0.05$, 0.01, 0.001) in percentage weight gain from the 12th to 21st day at the dose of 200 mg/kg. Likewise, co-administration with HEAG showed a significant decrease ($p < 0.05$ & 0.001) in % weight gain at the dose of 400 mg/kg from the 12th day to 21st day (Figure 1).

Effect on the percentage of body weight gain. Data are presented as mean ± SD ($n = 6$). Statistical analysis was performed using two-way ANOVA followed by Bonferroni's multiple comparison test. Two-way ANOVA revealed significant effects of treatment [F(4,200)=33.23, $p < 0.0001$], time [F(7,200)=321.9, $p < 0.0001$], and treatment × time interaction [F(28,200)=8.262, $p < 0.0001$]. * $p < 0.05$ and *** $p < 0.001$ indicate significant differences compared with the Normal Control group at the corresponding time points, whereas # $p < 0.05$, ## $p < 0.01$ and ### $p < 0.001$ indicate

significant differences compared with the OLZ group at the corresponding time points, as determined by Bonferroni's multiple comparison.

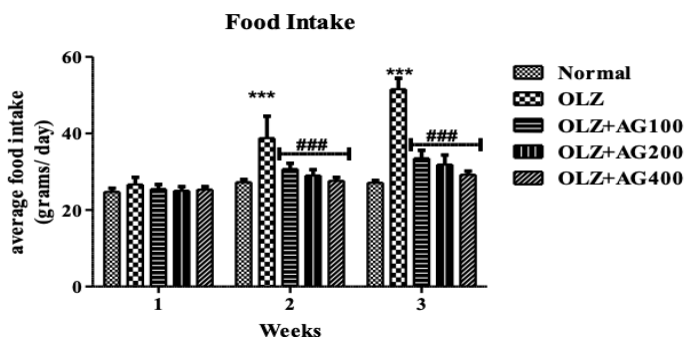
Figure 1: Effect on the percentage of weight gain



Effect on food intake

There was a significant increase ($p < 0.001$) in average food intake in the 2nd and 3rd week in an olanzapine-treated group compared to normal. Likewise, co-treatment with HEAG with olanzapine showed a significant decrease ($p < 0.001$) in food intake in the 2nd and 3rd week at the doses of 100, 200, and 400 mg/kg (Figure 2).

Figure 2: Effect on food intake

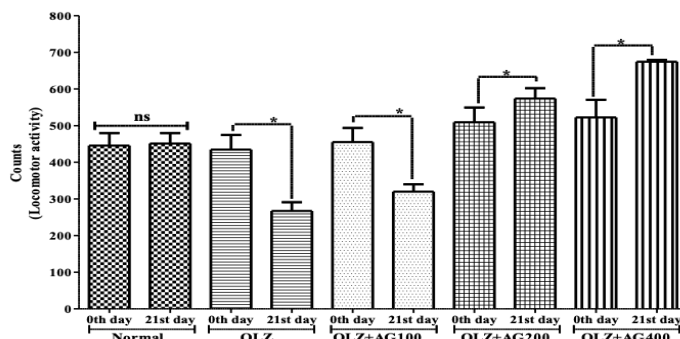


Data are presented as mean \pm SD ($n = 6$). Statistical analysis was performed using two-way ANOVA followed by Bonferroni's multiple comparison test. Two-way ANOVA revealed significant effects of treatment [$F(4,75)=101.2, p < 0.0001$], week [$F(2,75)=143.0, p < 0.0001$], and treatment \times week interaction [$F(8,75)=28.15, p < 0.0001$]. *** $p < 0.001$ indicates significant differences compared with the Normal Control group at the corresponding week, whereas ### $p < 0.001$ indicates significant differences compared with the OLZ group at the corresponding week.

Effect on locomotor activity

There was no significant increase or decrease in the locomotor activity on the 21st day compared to the 0th day in the normal group. However, there was a significant decrease ($p < 0.001$) in locomotor activity in olanzapine-treated groups on the 21st day compared to the 0th day. Similarly, there was a significant decrease ($p < 0.001$) in locomotor activity in AG-treated rats at a dose of 100 mg/kg. In contrast, there was a significant increase ($p < 0.001$) in the locomotor activity on the 21st day compared to the 0th day at the doses of 200 and 400 mg/kg (Figure 3).

Figure 3: Effect on locomotor activity

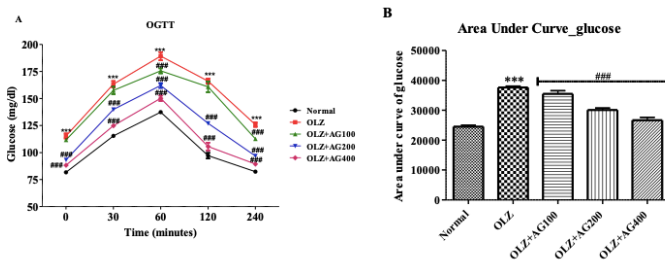


Data are presented as mean \pm SD ($n = 6$). Statistical analysis was performed using a paired t-test. ns indicates no significant difference between the 0th day and 21st day within the same group, whereas * $p < 0.001$ indicates a significant difference between the 0th day and 21st day within the same group.

Effect on the oral glucose tolerance test

There was a significant increase ($p < 0.001$) in blood glucose level from 0-240 min in an olanzapine-treated group compared to normal. Similarly, co-treatment with HEAG at the dose of 100 mg/kg showed a significant decrease ($p < 0.001$) compared to the olanzapine-treated group at 60 and 240 min. Further, co-treatment of HEAG with olanzapine at the doses of 200 and 400 mg/kg showed a significant decrease in blood glucose level at 0-240 min. Similarly, there was a significant increase ($p < 0.001$) in total area under the curve in an olanzapine-treated group compared to normal, which was significantly reversed ($p < 0.001$) with the co-administration of HEAG at all doses (Figure 4).

Figure 4: Effect on oral glucose tolerance test



Data are presented as mean \pm SD ($n = 6$). OGTT data were analyzed using two-way ANOVA followed by Bonferroni's multiple comparison test, whereas area under the curve (AUC) data were analyzed using one-way ANOVA followed by Dunnett's multiple comparison test. *** $p < 0.001$ indicates significant differences compared with the Normal Control group, whereas ### $p < 0.001$ indicates significant differences compared with the Olanzapine group.

There was a significant increase ($p < 0.001$) in total cholesterol, triglycerides, and low-density lipoprotein in the olanzapine-treated group compared to the normal. Similarly, co-administration of HEAG with olanzapine showed a significant decrease ($p < 0.01, 0.001$) in total cholesterol, triglycerides, and low-density lipoprotein at the doses 100, 200, and 400 mg/kg. In contrast, there was a significant decrease ($p < 0.001$) in high-density lipoprotein in olanzapine-treated groups compared to normal,

which was significantly reversed ($p < 0.01$, 0.001) at the doses of 100, 200, and 400 mg/kg HEAG (Table 1).

Table 1: Effect on lipid profile

Groups	Total Cholesterol	Triglycerides	High-density lipoprotein	Low-density lipoprotein
Normal	76.70±1.04	92.59±1.16	42.33±1.99	14.34±0.00
OLZ	93.01±0.76*	121.3±1.04*	24.21±1.75*	44.53±1.35*
OLZ+ AG100	87.69±1.87 #	117.8±2.11 #	27.66±0.61 #	36.48±1.94 #
OLZ+ AG200	83.5±3.07 ##	110.2±1.92 ##	28.60±0.86 #	32.85±3.46 ##
OLZ+ AG400	77.32±4.65 ##	95.82±1.68 ##	38.99±2.83 ##	19.13±6.84 ##

Data are analyzed using One-way ANOVA followed by the Dunnett test. All data are presented in mean±SD (n=6), * $p < 0.001$ compared to normal, # $p < 0.01$, ## $p < 0.001$ compared to Olanzapine

Discussion

The present study investigated the effect of hydroalcoholic extract of *Anethum graveolens* L. seeds against olanzapine-induced weight gain, hyperphagia, impaired glucose/lipid tolerance, and altered energy expenditure, in which administration of olanzapine (2mg/kg, i.p.) to female Sprague Dawley rats enhanced hyperphagia resulting in weight gain as reported previously (5).

Earlier reports reflect that the olanzapine-induced weight gain is associated with hyperphagia (18), which was also observed in the present study. Further, olanzapine-associated hyperphagia is linked to the 5-HT antagonist effect of olanzapine, which leads to increased weight gain (19). Previously, it was reported that *Anethum graveolens* L. seed extract stimulates 5-HT metabolism and reduces feed intake and body weight in rats (13). Hence, the observed decreased feed intake in olanzapine-induced obesity could be the outcome of stimulated 5-HT metabolism in the whole brain. Further, the function of serotonin in arousal and motor activity has been reported (20); hence, increased function of serotonergic function by *Anethum graveolens* L. seed extract (13) could be responsible for enhanced locomotor activity, which might have caused increased energy expenditure leading to decreased body weight.

Earlier, it has been suggested that the decreased locomotor activity in rats' model of olanzapine-induced weight gain, leading to reduced energy expenditure partially responsible for olanzapine-induced weight gain (21, 22). Similarly, in the present study, decreased locomotor activity was observed in the olanzapine-treated rats, reflecting the decreased energy expenditure. After the treatment with the *Anethum graveolens* L. seed extract showed a significant increase in the locomotor activity, this could be the outcome of enhanced energy expenditure.

Previous reports suggested that the decreased locomotor activity in olanzapine treatment (22-24) due to olanzapine-associated sedation could contribute to weight gain due to reduced energy expenditure (22). In the present study, a significant increase in locomotor activity was observed in AG coadministered animals, which reflects the increased energy expenditure, which could be the outcome of 5-HT metabolism by *Anethum graveolens* L. (13)

as the serotonergic control is directly involved in metabolic homeostasis (19).

Oral glucose tolerance test measures the ability to regulate the glucose metabolism via the process of glycolysis or glucose uptake in the presence of insulin (25); also reflects the pancreatic β sensitivity for exogenous glucose (26) and also possesses an important role in managing diabetes (14). Additionally, supplementation of *Anethum graveolens* L. (dill) has improved the insulin function and metabolic status in patients with type 2 diabetes (27). Similarly, in the present study, treatment with *Anethum graveolens* L. seed extract showed a significant decrease in total area under the curve of glucose during an oral glucose tolerance test. Further, previous reports have reflected the mild hyperglycemia in olanzapine-treated animals (5); which was also observed in the present study; ameliorated after the treatment with the *Anethum graveolens* L. seed extract, which could be the outcome of improved glucose metabolism and enhanced pancreas β -cell sensitivity.

Multiple experimental studies reflected the deregulated lipid metabolism in olanzapine-treated rats (5, 28, 29), which was also observed in the present study. There was a significant increase in total cholesterol, triglycerides, and low-density lipoprotein. In contrast, there was a significant decrease ($p < 0.001$) in the HDL level compared to normal. Further previous reports via the clinical trial also reflected the improved lipid metabolism by *Anethum graveolens*, which ameliorates hyperlipidaemia (30). Peroxisome proliferator-activated receptors are ligand-activated transcription factors from the nuclear hormone receptor superfamily and are expressed in the kidney, liver, muscle, heart, and liver where the β -oxidation principally occurs (31). Beta-oxidation of free fatty acid is the prime basis of lipid metabolism and its regulation (32), which is one of the deregulated pathways in obesity (33) and its associated pathogenesis. Previously, it has been reported that dill seed extract improves abnormalities in lipid metabolism through peroxisome proliferator-activated receptor- α (PPAR- α) activation (34). Hence, in the present study, the improved lipid metabolism in olanzapine-induced obesity could be the outcome of PPAR-mediated lipolysis.

Conclusion

In conclusion, the present study reported the beneficial effect of *Anethum graveolens* L. seed extract to manage olanzapine-induced obesity via improved lipid and glucose metabolism, followed by reversing the hyperphagia. However, there is still a necessity to confirm the present findings via the quantification of suitable biomarkers that are associated with olanzapine-induced obesity.

Conflict of interest: The authors declare that they have no conflict of interest.

Ethical statement: This experiment has been performed after receiving ethical clearance from the IAEC at KLE College of Pharmacy, Belagavi. Resolution No. KLE COP/CPCSEA-Reg.No.221/Po/Re/S/2000/CPCSEA.Reg.28-12/10/2019.

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