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# Effect of ursolic acid on olanzapine induced weight gain in Sprague Dawley rats

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Antipsychotics used in the treatment of schizophrenia are known to cause weight gain. Phytoconstituents are used to manage such drug-induced weight gain. Ursolic acid is a pentacyclic triterpenoid commonly present in many herbs and fruits which are used in daily life. The effect of ursolic acid on antipsychotic-induced weight gain is not clear. Here, we investigated the effect of ursolic acid on olanzapine-induced weight gain in rats. Sprague-Dawley (SD) rats were divided into six different groups *viz.*, normal control, olanzapine control, betahistine (10 mg/kg), and ursolic acid 10, 20 and 40 mg/kg treated groups. All the drugs were administered once daily for 28 days orally. Except for the normal control group, all other animals were treated with olanzapine 4 mg/kg intraperitoneally to induce weight gain. During the experiment, animal's behaviour variations were monitored at regular intervals. At the end of the experiment, blood sample was collected from all the experimental animals for biochemical analysis. Part of the brain, liver, heart, lung and kidney tissues were excised from the sacrificed animals and preserved in neutral formalin for histopathological studies. Ursolic acid showed a significant reduction in olanzapine-induced body weight gain on the rats. Increase in locomotor activity was also observed with the treatment of ursolic acid. Compared to ursolic acid, betahistine showed better tolerance against olanzapine-induced body weight gain.

**Keywords**: Antipsychotics, Depression, Schizophrenia

Antipsychotics are major tranquilizers used in the treatment of schizophrenia, bipolar disorder, dementia, unipolar depression, obsessive-compulsive disorder, etc[1]. The adverse effects of antipsychotics include impaired memory, extrapyramidal effects, weight gain and metabolic dysfunction[2]. Weight gain is often the most common and visible side effect of atypical antipsychotic agents and it causes serious health risks[3]. The atypical antipsychotics such as clozapine and olanzapine have the most weight gaining potential when compared with other antipsychotic agents[4]. Olanzapine is a thienobenzodiazepine and acts as antagonist at $5HT_{2A}$ and $D_2$ receptor[5]. While the corticostriatal pathways are responsible for therapeutic action of olanazepine, the actions on nigrostriatal pathway, and antihistaminic and antiserotonergic properties probably cause adverse effects[6]. Many of the plant phytoconstituents including ursolic acid found to have preventive effect against drug-induced weight gain.

Ursolic Acid is a pentacyclic triterpenoid, widely distributed in herbs[7]. In many studies, ursolic acid showed a beneficial effect against diet or drug-induced weight gain[8-10]. Ursolic acid has significant nitration with muscarinic, dopaminergic, peroxisome proliferator-activated receptor (PPAR)-α and, etc. receptor sites[11,12]. Earlier studies have reported that PPAR-α agonist reverses the increased levels of triglycerides and insulin resistance caused by antipsychotics like chlorpromazine, clozapine, and ziprasidone[13,14]. It is reported that ursolic acid also has the ability to increase protein kinase B (Akt) activity in muscles that involve in multiple cellular processes such as cell apoptosis, cell proliferation, cell migration, transcription, glucose metabolism, and increased fat burning. Akt, specifically located in skeletal muscles which elevate the energy expenditure, reduces adiposity and blood glucose and impart resistance to glucose intolerance, diet-induced weight gain, and fatty liver disease[15]. The effect of ursolic acid on atypical antipsychotic agent-induced weight gain remains unclear. Hence, in the present study, we explored the effect of ursolic acid on olanzapine-induced weight gain and associated

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metabolic and behavioural variations and compared it with betahistine, a known inhibitor of antipsychotic-induced weight gain, related metabolic and behavioural variations.

## Materials and Methods

### Chemicals

Ursolic acid and olanzapine were purchased from Sigma-Aldrich, USA. Betahistine Dihydrochloride (Betaserc®) was purchased from a retail pharmacy. Ursolic acid was suspended with 0.05% w/v of carboxymethyl cellulose (CMC). Olanzapine was dissolved in 0.1 N hydrochloric acid and pH was adjusted to ~5.5 (using 0.1 N NaOH) and the final volume was adjusted with distilled water[16].

### Animals

Healthy, adult, female Sprague-Dawley (SD) rats, weighing 150±10 g obtained from the Central Animal house, AIMST University, Malaysia were housed in large, spacious poly acrylic cages at ambient room temperature (22-25°C) with 12 h light/dark cycle and fed with water, and normal rats pellet diet *ad libitum*. Prior approval was obtained from AIMST University Human and Animal Ethics Committee (AUHAEC1/FOP/SP/2015) to carry out the study, and conducted according to University Animal Research Review Panel guidelines.

### Effect of ursolic acid on olanzapine-induced weight gain

The rats were divided into six groups of six animals. Animals in group I was considered as normal control and they were treated with CMC. Group II animals were treated with olanzapine (4 mg/kg/day) and group III was treated with betahistine (10 mg/kg/day) along with olanzapine (4 mg/kg/day). Animals in group IV to VI were treated with ursolic acid 10, 20 and 40 mg/kg/day, respectively, along with olanzapine (4 mg/kg/day)[17]. Olanzapine was administered intraperitoneally and ursolic acid was administered orally every day for 28 days.

During the study, body weight changes, food and water intake variations and, behavioural alterations were monitored at regular intervals. At the end of the study, animals were mildly anesthetized with diethyl ether and one ml of blood was collected for biochemical analysis, then they were sacrificed by cervical dislocation. The brain, lung, heart, liver, kidney, reproductive organs and peritoneal adipose tissues were collected and absolute organ weight was measured. The body weight of each rat in each group was recorded at regular intervals.

Daily food and water intake was measured in each group and compared with that of control.

### Behavioural analysis

The locomotor activity, anxiety levels, learning and memory activities, and grip strength were evaluated at regular intervals.

*Locomotor activity*

The mobility of rats were recorded in a rodent activity cage (actophotometer [manufactured by Jainsons (India) Regd. Haryana, India]) equipped with acrylic cage and six built-in photo sensor and digital counters beams on both x and y horizontal axis. The activity of the individual rat was monitored at room temperature for 5 min.

*Learning and memory* (*water maze test*)

Water navigation test was employed to assess learning and memory parameters and, escape latency time (ELT) to evaluate the spatial learning and memory functions. The water maze consists of circular tank with 90 cm diameter and wall 20 cm above the water level with 25°C water. A square platform (10×5 cm) is hidden 2 cm below the water level. Training had been taken place for 3 consecutive days, with a 4 consecutive trials/day for each experimental rat at the inter-trial interval of 30 min. The tank was divided into 4 equal quadrants and trail was started from one of four assigned polar positions with different sequence each day. The latency was measured by observing the time to reach the platform by the animal. Any animal fails to reach the platform in any trial within 3 min will be excluded from the study[18].

*Anxiogenic effects* (*elevated plus maze*)

The rodent elevated plus maze consists of two open arms and two enclosed arms with the dimension of 50×10×60 (L-W-H) cm. Two open and enclosed arms are opposite to each other and maze is elevated to a height of 60 cm. Prior to the experiment and at 14$^{th}$ and 28$^{th}$ day of the experiment, the rat was placed in the center of the maze, facing one of the enclosed arms and monitored for 10 min. The number of entries to each arm and time spent in each arm were recorded. The experiment was conducted at a quiet environment[19].

*Wire grip strength*

This test employed to assess the skeletal muscle function in rodents. The apparatus consisted of a metallic wire (90 cm length, 4 mm in diameter), fixed horizontally between two retort stands and 60 cm above a smooth surface[18]. The rats were forced to hold

the middle of the wire. Training was conducted for three consecutive days. Control rats as well as olanzapine administered rats were observed for the duration taken by rats to support their weight holding on the metal string attached. Each rat was subjected to 5 trials with at least 10 min rest period in between tests[20]. The latency to fall from the wire was noted.

#### Biochemical parameters

At the end of the study, one ml of the blood was collected from all the animals through retro-orbital sinus puncture by using a plain glass tube. The serum was separated by centrifugation at 3000 RPM for 20 min at 4°C, and stored at −80°C until further biochemical analysis.

The serum levels of alkaline Phosphatase (ALP), glucose, aspartate transaminase (AST), alanine transaminase (ALT), total protein, urea, creatinine, total cholesterol (TC), triglyceride (TG), and high density lipoprotein-cholesterol (HDL) were analyzed using biochemical analyzer (Reflotron Plus System, Hoffmann-La Roche, USA). The Non-HDL, LDL, HDL ratio, VLDL and Atherogenic dyslipidemia [AD] were calculated using following formula TC-HDL; TC/1.19 + TG/1.9 – HDL/1.1 – 38; [HDL-cholesterol/TC-HDL-cholesterol] ×100; LDL/5 and log (TG/HDL-C)[21-23].

#### Histopathological analysis

Part of the liver and kidney samples from normal control, olanzapine control, betahistine and ursolic acid 40 mg/kg groups were preserved in 10% neutral formalin for histopathological analysis. The brain, liver, and kidney tissue was embedded in paraffin after being dehydrated in alcohol and subsequently cleared with xylene. Paraffin-embedded tissue blocks were prepared and five-micrometer thickness of section was taken with Microtome (Thermo Electron Corporation, England) for routine histopathology. Briefly, the sections for histopathology was placed on an albuminized glass slide, deparaffinized, rehydrated and stained with routine hematoxylin and eosin (H&E). After mounting with DPX and coverslip the slides were examined under a light microscope and reporting was done.

#### Statistical analysis

The mean ± standard error of the mean (n = 6) values was calculated for each group except group III and IV (n = 5). It was found that one animal from Group III and IV were dead on the 6$^{th}$ day of the experiment. Statistical differences among the groups were determined using One-way ANOVA followed by Tukey's *post-hoc* test. $P$ <0.05 is considered as significant.

## Results

#### Effect of ursolic acid on food and water intake

The group treated with olanzapine and olanzapine + ursolic acid 10 mg showed a significant increase in food intake while the group treated with olanzapine + ursolic acid 40 mg did not show any significant alteration in regular food intake when compared to control. Rats treated with olanzapine and olanzapine + ursolic acid 10 mg showed a significant increase in food intake from 14$^{th}$day onwards when compared with that of control. The effect of ursolic acid on olanzapine-induced changes in food intake of rats is summarized in Fig. 1. Significant changes in regular water intake were not observed with any of the treated group when compared to control.

#### Effect of ursolic acid on olanzapine-induced weight gain

The animals treated with olanzapine alone were found to have significant weight gain. The animals treated with olanzapine plus ursolic acid 10 mg/kg a showed significant increase in body weight compared to other groups. While the rats treated with olanzapine + ursolic acid 40 mg showed a significant decrease in olanzapine-induced weight gain when compared to olanzapine control. The rats treated with olanzapine + betahistine showed no significant increase in body weight. The effect of ursolic acid on olanzapine-induced weight gain on rats is summarized in Table 1.

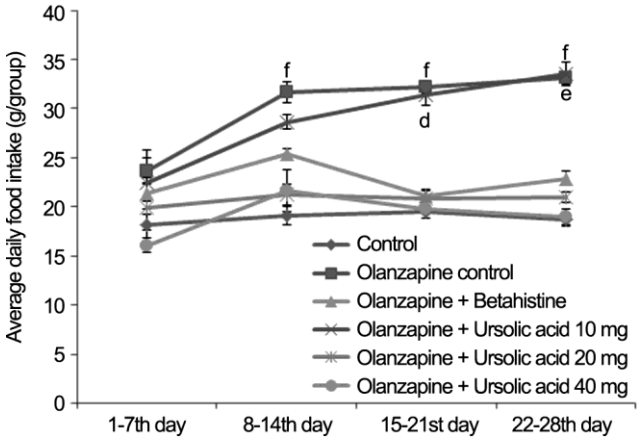


Fig. 1 — Effect of ursolic acid on olanzapine-induced changes in food intake. [All the values are mean ± SEM (n=6, except olanzapine + betahistine [n=5] and olanzapine + ursolic acid 10 mg [n=5] treated group). $^{d}P$ <0.05; $^{e}P$ <0.01 and $^{f}P$ <0.001 compare to 1-7$^{th}$ day. (One-way ANOVA followed by Tukey *post-hoc* test). SEM: Standard error of the mean].

Table 1 — Effect of ursolic acid on olanzapine-induced body weight gain of rats

| Treatment | Body weight (g) | | | | | % changes (at the end of study) |
|---|---|---|---|---|---|---|
| | Pre-study day | 7th day | 14th day | 21st day | 28th day | |
| Control | 152.50±3.74 | 153.50±4.06 | 156.67±3.29 | 158.83±2.39 | 162.67±2.40 | 6.87±2.06 |
| Olanzapine control | 149.33±3.99 | 154.17±3.85 | 165.00±4.07 | 168.33±4.18 | 179.17±3.31# | 20.13±1.40 |
| Olanzapine + Betahistine | 150.20±3.32 | 152.80±2.37 | 154.80±2.59 | 155.80±2.71 | 157.00±1.87*** | 4.66±1.41 |
| Olanzapine + Ursolic acid 10 mg | 149.00±2.58 | 152.40±3.20 | 156.60±2.59 | 159.80±2.47 | 166.40±2.30* | 11.71±0.62 |
| Olanzapine + Ursolic acid 20 mg | 149.17±2.41 | 149.33±1.50 | 151.67±1.63* | 152.33±2.01** | 158.00±1.77*** | 5.99±1.09 |
| Olanzapine + Ursolic acid 40 mg | 148.83±2.81 | 150.17±3.16 | 149.67±3.13* | 149.50±2.95*** | 149.17±2.74*** | 0.25±0.88 |

[All the values are mean ± SEM (n=6, except olanzapine + betahistine [n=5] and olanzapine + ursolic acid 10 mg [n=5] treated group). **P* <0.05, ***P* <0.05, ****P* <0.05 compare to olanzepine treated group; #*P* <0.05 compare to control group (One-way ANOVA followed by Tukey *post-hoc* test). SEM: Standard error of the mean, SD: Sprague-Dawley]

Table 2 — Effect of ursolic acid on locomotor activity, escape latency time and grip strength of rats

| Treatment | Locomotion (Unit/5 min) | | | Escape latency time in seconds | | | Wire screen holding time in seconds | | |
|---|---|---|---|---|---|---|---|---|---|
| | Pre-study day | 14th day | 28th day | Pre-study day | 14th day | 28th day | Pre-study day | 14th day | 28th day |
| Control | 106.67±10.21 | 93.67±28.21 | 101.50±11.11 | 47.17±5.26 | 48.00±4.68 | 43.67±3.06 | 26.00±3.43 | 27.50±2.73 | 28.83±4.10 |
| Olanzapine control | 101.50±24.30 | 90.17±22.58 | 10.67±2.81# | 44.67±9.66 | 31.67±8.58 | 25.50±8.95@ | 27.33±7.70 | 20.33±5.28 | 19.50±5.83 |
| Olanzapine+Betahistine | 91.40±17.66 | 114.60±17.12 | 101.20±6.51 | 44.80±4.17 | 44.80±4.93 | 27.60±4.05 | 30.00±12.41 | 48.20±13.19 | 59.80±14.47* |
| Olanzapine+Ursolic acid 10 mg | 96.20±11.56 | 99.80±28.14 | 68.00±16.55* | 46.33±3.73 | 22.00±5.38 | 35.00±8.90 | 29.83±8.77 | 33.60±9.27 | 35.60±11.02 |
| 20 mg | 101.17±11.29 | 73.83±14.42 | 101.50±11.11 | 44.00±8.99 | 36.33±7.84 | 29.67±6.22 | 31.00±4.32 | 36.17±7.36 | 45.83±7.87 |
| 40 mg | 106.33±8.39 | 97.33±14.90 | 154.00±16.68** | 44.83±8.90 | 30.83±7.89 | 23.00±4.41 | 28.83±6.85 | 41.00±6.94 | 48.17±6.12 |

[All the values are mean ± SEM (n=6, except olanzapine + betahistine [n=5] and olanzapine + ursolic acid 10 mg [n=5] treated group). **P* <0.05; ***P* <0.001 compare to olanzepine treated group; #*P* <0.001 compare to control group (One-way ANOVA followed by Tukey *post-hoc* test). @Value of two animals (Only two animals reached platform, remaining animals failed to reach platform). SEM: Standard error of the mean]

**Effect of ursolic acid on Behaviour**

*Effect of ursolic acid on locomotion*

Locomotion is considered as one of the indices for mental alertness. The rats treated with olanzapine showed a decrease in locomotor activity by 11.16% on the 14th day and 88.17% on the 28th day when compared to initial values. The rats treated with ursolic acid 20 and 40 mg/kg showed significant dose-dependent increases in locomotor activity throughout the study when compared to the olanzapine treated group. The effect of ursolic acid on motor coordination on rats is summarized in Table 2. Throughout the study, betahistine administered animals did not show any alteration when compared to the pre-study locomotor activities.

*Effect of ursolic acid on Escape latency time*

Effect of ursolic acid on water navigation of rats is summarized in Table 2. In post training, ursolic acid treated animals showed decreased ELT compared with that of control.

*Effect of ursolic acid on grip strength*

Throughout the study, the rats treated with olanzapine showed a decrease in their grip strength (−25.61% on the 14th day and −4.08% on the 28th day). The rats treated with betahistine showed a significant increase in their grip strength. The rats treated with ursolic acid also showed an increase in grip strength, but the values are not significant. The effect of ursolic acid on olanzapine on grip strength of rats is shown in Table 2.

*Anxiogenic effects ursolic acid*

The normal control animals did not show any alterations in behaviour whereas betahistine and ursolic acid treated animals showed significant alterations in anxiogenic behaviour (Fig. 2). The rats administered with olanzapine showed an increased number of entries in dark arm and the rats treated with betahistine showed increased time spent in dark compared with that of pre-study values. The rats treated with ursolic acid showed decreased duration of stay and number of entries in dark.

**Effect of ursolic acid on absolute and relative organ weights**

Effects of ursolic acid on absolute and relative organ weight of experimental rats were summarized in Table 3. The rats treated with olanzapine showed a significant increase in the absolute organ weight of brain, kidney, and uterus when compared to control. Betahistine and ursolic acid 10 mg/kg treated animals showed a significant increase in lung weight when compared to control. Olanzapine treated animals showed a significant increase of relative uterus weight when compared to control. Betahistine and ursolic acid 10 mg/kg treated animals showed a significant increase in lung's weight when compared to the olanzapine treated group.

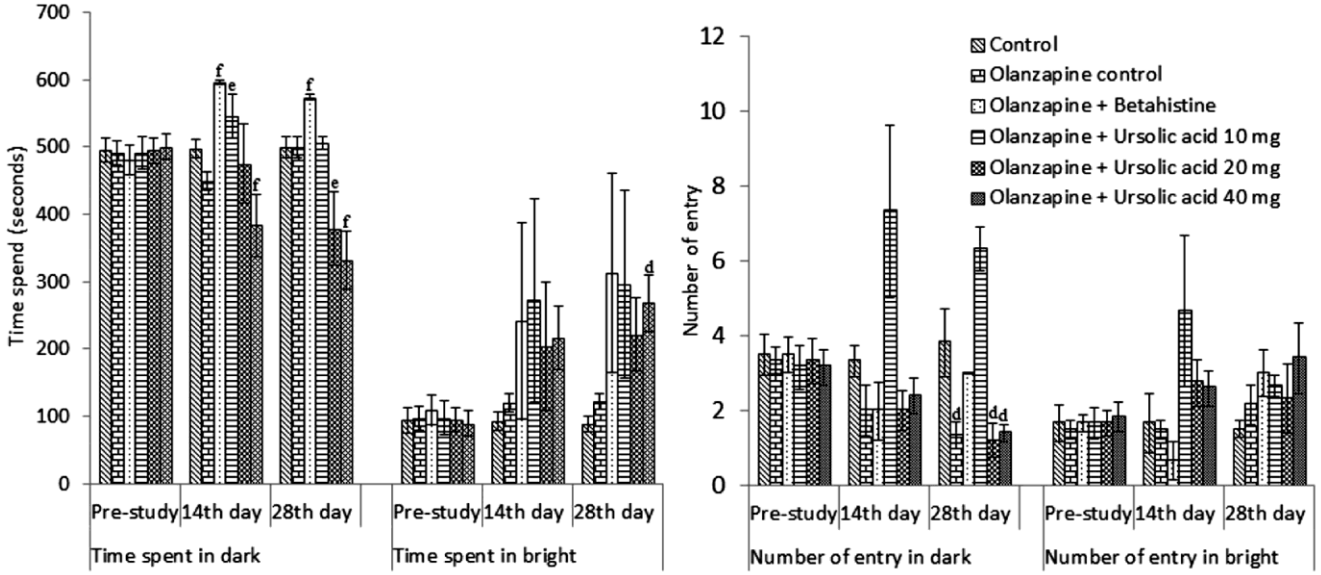


Fig. 2 — Effect of ursolic acid on anxiety (elevated plus maze) of rats. [All the values are mean ± SEM (n=6, except olanzapine + betahistine [n=5] and olanzapine + ursolic acid 10 mg [n=5] treated group). $^{d}P$ <0.05; $^{e}P$ <0.01 and $^{f}P$ <0.001 compare with that of pre-study values (One-way ANOVA followed by Tukey *post-hoc* test).SEM: Standard error of the mean].

Table 3 — Effect of ursolic acid on (A) absolute; and (B) relative organ weight of rats

| Treatment | Brain | Lung | Heart | Liver | Kidney (Left) | Kidney (Right) | Kidney (Mean) | Uterus |
|---|---|---|---|---|---|---|---|---|
| | Absolute organ weight (g) | | | | | | | |
| Control | 1.71±0.06 | 1.54±0.06 | 0.57±0.03 | 5.96±0.33 | 0.32±0.02 | 0.31±0.02 | 0.63±0.04 | 2.62±0.14 |
| Olanzapine control | 2.01±0.08$^{\#\#}$ | 1.66±0.05 | 0.65±0.03 | 6.44±0.17 | 0.38±0.01$^{\#}$ | 0.37±0.01$^{\#}$ | 0.75±0.03$^{\#}$ | 1.83±0.15$^{\#}$ |
| Olanzapine+Betahistine | 1.68±0.08** | 1.24±0.09$^{\#}$*** | 0.57±0.04 | 5.77±0.19 | 0.30±0.01** | 0.29±0.01** | 0.58±0.03** | 1.94±0.14 |
| Olanzapine+Ursolic acid 10 mg | 1.65±0.04** | 1.12±0.06$^{\#\#\#}$*** | 0.56±0.04 | 6.22±0.56 | 0.35±0.01 | 0.35±0.01 | 0.70±0.03 | 1.98±0.19 |
| Olanzapine+Ursolic acid 20 mg | 1.52±0.04*** | 1.42±0.03 | 0.53±0.02 | 5.13±0.10 | 0.30±0.01** | 0.29±0.01** | 0.59±0.02** | 1.80±0.21$^{\#}$ |
| Olanzapine+Ursolic acid 40 mg | 1.53±0.04*** | 1.33±0.04** | 0.57±0.07 | 5.57±0.36 | 0.30±0.01** | 0.30±0.01** | 0.59±0.02** | 2.04±0.20 |
| | Relative organ weight (g) | | | | | | | |
| Control | 1.05±0.03 | 0.94±0.03 | 0.35±0.01 | 3.65±0.16 | 0.20±0.01 | 0.19±0.01 | 0.39±0.02 | 1.61±0.10 |
| Olanzapine control | 1.13±0.06 | 0.92±0.02 | 0.36±0.02 | 3.61±0.14 | 0.21±0.01 | 0.21±0.00 | 0.42±0.01 | 1.02±0.07* |
| Olanzapine+Betahistine | 1.07±0.04 | 0.79±0.06$^{\#}$ | 0.36±0.03 | 3.67±0.12 | 0.19±0.01 | 0.18±0.01 | 0.37±0.01 | 1.24±0.10 |
| Olanzapine+Ursolic acid 10 mg | 0.99±0.03 | 0.67±0.03$^{\#\#}$** | 0.34±0.02 | 3.73±0.31 | 0.21±0.01 | 0.21±0.01 | 0.42±0.01 | 1.20±0.12 |
| Olanzapine+Ursolic acid 20 mg | 0.96±0.03 | 0.90±0.02 | 0.34±0.01 | 3.25±0.07 | 0.19±0.01 | 0.19±0.01 | 0.38±0.01 | 1.14±0.14 |
| Olanzapine+Ursolic acid 40 mg | 1.03±0.03 | 0.89±0.03 | 0.38±0.05 | 3.73±0.21 | 0.20±0.00 | 0.20±0.00 | 0.40±0.01 | 1.37±0.13 |

[All the values are mean ± SEM (n=6, except olanzapine + betahistine [n=5] and olanzapine + ursolic acid 10 mg [n=5] treated group). *$P$ <0.01; **$P$ <0.001 compared to olanzepine treated group; $^{\#}P$ <0.05, $^{\#\#}P$ <0.01 and $^{\#\#\#}P$ <0.001 compared to control group (One=way ANOVA followed by Tukey *post-hoc* test). SEM: Standard error of the mean]

**Effects of ursolic acid on serum biochemical parameters**

The animals treated with olanzapine showed significantly increased levels of serum glucose and creatinine when compared with that of the control group. Animals treated with betahistine and ursolic acid showed prevention of olanzapine-induced raise in serum glucose (Fig. 3A) and creatinine (Fig. 3B) levels. Olanzapine significantly increased the levels of triglycerides, LDL, VLDL and AD compared to that of control, whereas betahistine and ursolic acid prevented the olanzapine-induced raise in these parameters. Effect of ursolic acid on serum biochemical parameters and lipids profile are summarized in Figs 3 and 4.

**Effects of ursolic acid on histology of organs**

Histopathological findings are presented in Table 4 and Fig. 5. Olanzapine treated animals showed mononuclear infiltration, dilated alveoli and vascular congestion in lungs (Fig. 5 A-C) whereas normal features and mild lymphocyte infiltration were observed with betahistine (Fig. 6A) and ursolic acid (40 mg/kg) treated animals, respectively. Fatty changes and degeneration of hepatocytes in the liver, cardiac

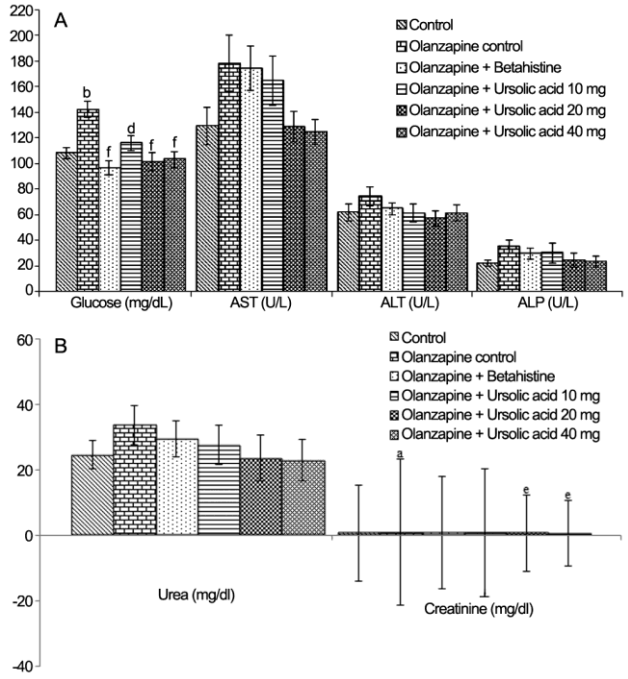


Fig. 3 — (A) Effects of ursolic acid on serum biochemical parameters of rats; and (B): Effects of ursolic acid on serum biochemical parameters of rats. [All the values are mean ± SEM (n=6, except olanzapine + betahistine [n=5] and olanzapine + ursolic acid 10 mg [n=5] treated group). $^{a}P$ <0.05 and $^{b}P$ <0.01 compared to control group; and $^{d}P$ <0.05, $^{e}P$ <0.01 and $^{f}P$ <0.001 compared to olanzepine treated group (One-way ANOVA followed by Tukey *post-hoc* test). AST: Aspartate aminotransferase, ALT: Alanine aminotransferase, ALP: Alkaline phosphatase and SEM: Standard error of the mean].

congestion in heart and severe tubular cell degeneration in kidney were observed in olanzapine treated animals (Fig. 5 D-F) whereas betahistine and ursolic acid reversed these effects (Fig. 6 A & B) and Fig. 7.

## Discussion

Ursolic acid at 20 and 40 mg/kg has significantly resisted the olanzapine-induced weight gain and its effect was comparable with that of betahistine. This suggests that ursolic acid inhibits olanzapine-induced

Table 4 — Effects of ursolic acid on histology of organs

| Treatment | Organ | Findings |
|---|---|---|
| Control (normal) | Brain, Lung, Heart, Liver, Kidney | Normal |
| Olanzapine control (4 mg/kg) | Brain | Normal |
| | Lung | Mononuclear cell infiltration, dilated alveoli and vascular congestion. |
| | Heart | Mild cardiac congestion |
| | Liver | Mild degeneration of hepatocytes and fatty changes |
| | Kidney | No changes in glomeruli and sever tubular cell degeneration |
| Olanzapine + Betahistine (10 mg/kg) | Brain, Lung | Normal |
| | Heart | No evidence of necrosis or myocardial toxin |
| | Liver | Mild degeneration of hepatocytes |
| | Kidney | No changes in glomeruli and mild tubular cell degeneration |
| Olanzapine +Ursolic acid (40 mg/kg) | Brain, Kidney | Normal |
| | Lung | Mild lymphocyte infiltration |
| | Heart | No evidence of necrosis. |
| | Liver | No evidence of degeneration of hepatocytes |

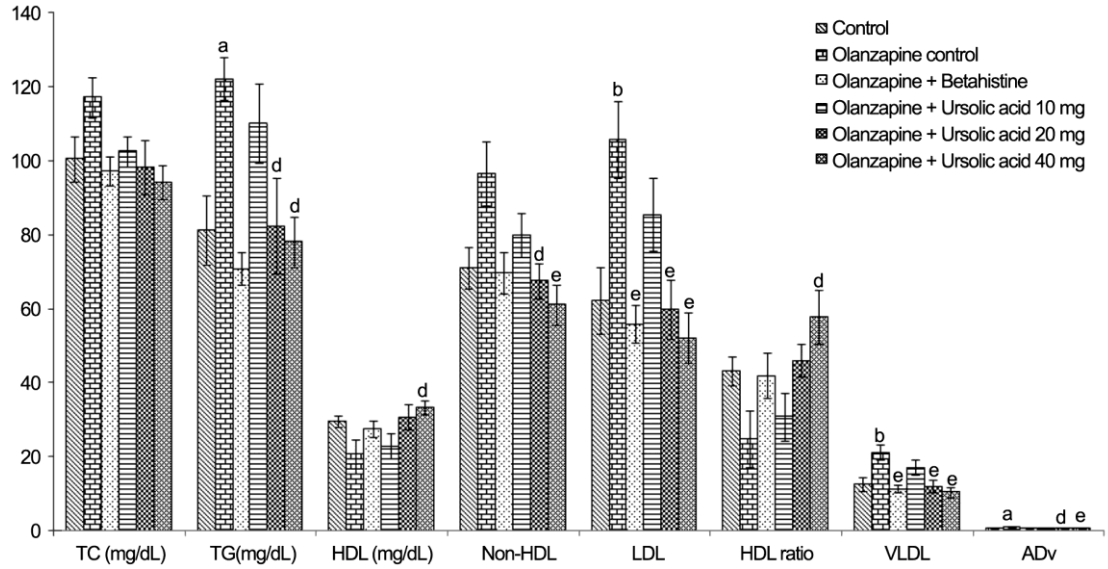


Fig. 4 — Effects of ursolic acid on plasma lipid profile of rats. [All the values are mean ± SEM (n=6, except olanzapine + betahistine [n=5] and olanzapine + ursolic acid 10 mg [n=5] treated group). $^{d}P$ <0.05; $^{e}P$ <0.01 compare to olanzepine treated group; $^{a}P$ <0.05 and $^{b}P$ <0.01 compare to control group (One-way ANOVA followed by Tukey *post-hoc* test)] TC: Cholesterol, TG: Triglycerides, HDL: High-density lipoprotein, LDL: Low-density lipoprotein, LDL: Low-density lipoprotein, AD: Atherogenicdyslipidemia, SEM: Standard error of the mean].

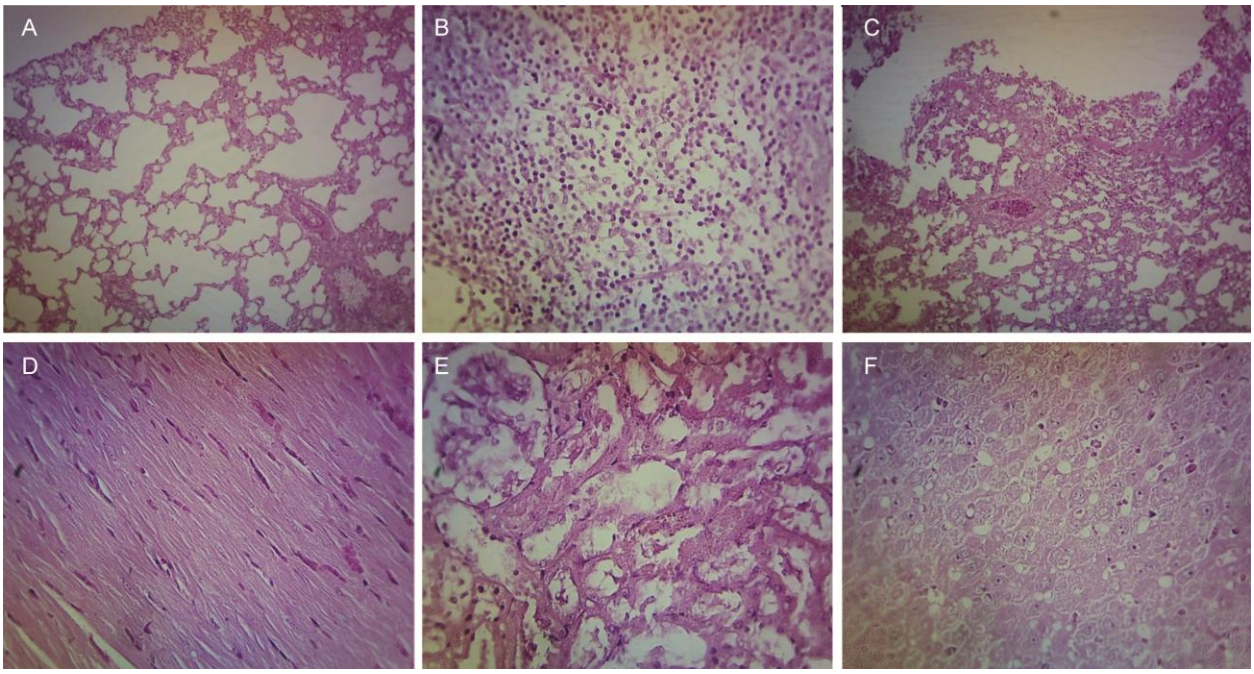


Fig. 5 — Histological features of various organs of rat treated with olanzapine 4 mg/kg. Section from lung of olanzapine 4 mg/kg treated animals shows (A) dilated alveoli; (B) infiltration of mononuclear cell; (C) vascular congestion; (D) heart showing mild cardiac congestion; (E) kidney showing sever tubular cell degeneration; and (F) liver showing mild degeneration of hepatocytes fatty changes. H&E, 400X.

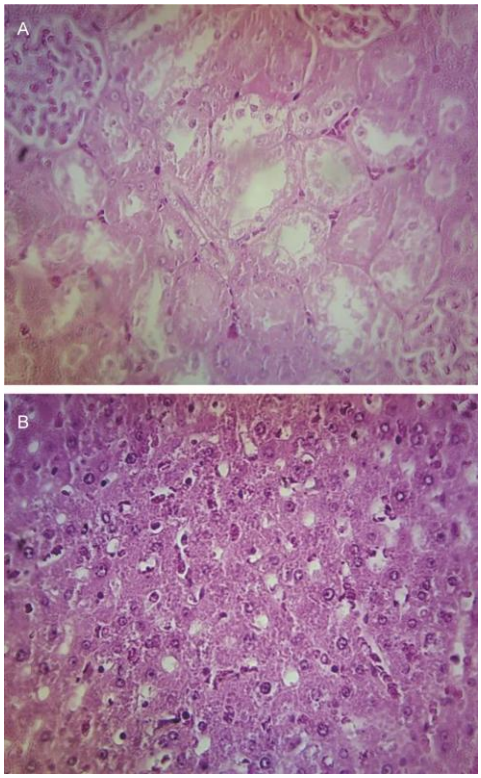


Fig. 6 — Histological features of various organs of rat treated with betahistine 10 mg/kg. (A) Section from kidney of betahistine treated animals showing mild tubular cell degeneration; and (B) liver showing mild degeneration of hepatocytes. H&E, 400X.

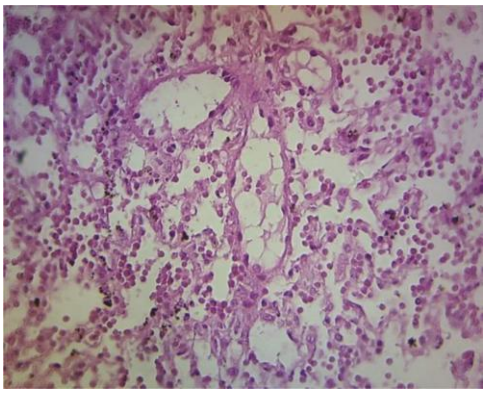

Fig. 7 — Section from lungs of ursolic acid 40 mg/kg treated animals shows mild lymphocyte infiltration. H&E, 400X.

weight gain. In some of the previous studies, betahistine, an $H_1$ agonistic or $H_3$ antagonist showed a beneficial effect against antipsychotics-induced weight gain. Lian et al reported that betahistine at 2.7 mg/kg (t.i.d.) showed significant weight reduction in the rats co-administered with olanzapine (1.0 mg/kg; t.i.d.) for 2 weeks[24]. In another study, betahistine reduced olanzapine-induced body weight by ~45%[25]. In humans, betahistine showed a significant weight reduction when it was co-administered with olanzapine (10 mg/day) in first-episode schizophrenia patients for 6 weeks[26]. These studies clearly showed

that partial histamine agonist/antagonist has a beneficial effect on the reduction of olanzapine-induced weight gain. The effect of ursolic acid on olanzapine-induced weight gain was compared with betahistine.

A pilot study of 'olanzapine-induced weight gain' was carried out in both the genders of SD rats. In the pilot study, both gender of animals received 1.0 mg/kg intraperitoneal injection of olanzapine for two weeks. At the end of the study, it was found that the male rats did not respond to olanzapine as they did not show any significant increase in regular food intake and olanzapine-induced weight gain when compared to control. Whereas female rats showed a significant increase in regular food intake and weight gain compared to control. Hence, further studies were carried out with female SD rats. Albaugh *et al*., also studied metabolic effects of olanzapine in rodents and found that A/J mice, C57B1/6J mice, and male SD rats are not suitable to mimic the human pathological conditions of olanzapine-induced metabolic alterations and weight gain. From there study it is exhibited that, olanzapine-induced weight gain in female but not in male SD rats[27]. In human also, it was suggested that olanzapine caused weight gain which is mediated by increased food intake and lack of physical activity[28]. Interaction of olanzapine with monoaminergic receptors and the effect of olanzapine administration on the regulation of hypothalamic mechanisms lead to hyperphagia, weight gain, and metabolic changes[29].

The animals treated with olanzapine showed significant increases in regular food intake and an increase in body weight when compared to normal animals. No significant changes in water intake were observed during the study. Murashita *et al.*, reported that, olanzapine increases appetite by stimulating secretion of ghrelin in ghrelinergic cells of gastrointestinal tract, which results in an increase in food intake and weight gain[30]. The food intake and olanzapine's effect on body weight was effetely resisted by ursolic acid. This may be due to the antisecretory effect or hyposecretion of ghrelin by ursolic acid[10]. Olanzapine increased water intake, but the values are not significant. Fell *et al*.[31] found that olanzapine increases regular weight gain, food and water intake, abdominal fat significantly. Ursolic acid inhibits olanzapine-induced weight gain by enhancing skeletal muscle insulin (IGF-I) signaling, activating Akt (reduces weight gain and elevate energy expenditure), reducing adiposity and blood glucose level[15].

Olanzapine found to have increased animals' body weight and reduced locomotion, immobilization time, grip strength and increased level of anxiety, lipids level and, glucose level whereas ursolic acid inhibits all these olanzapine-induced metabolic effects. This indicates ursolic acid has significant antianxiety, anti-hyperlipidemic and anti-hyperglycemic effects. Liu *et al*.[32] studied the effect of olanzapine on MK-801 induced cognitive deficits and found that, the animals treated with olanzapine + MK-801 decreased locomotor activity in the first 10 min of the test (results are not statistically significant) and olanzapine rescued animals from MK-801 induced cognitive deficits. Reduction in locomotor activity effects of olanzapine may be due to the reduction of accumbens nucleus (Acb) GABAergic activity[33].

Yuliang *et al.*[34] reported the hypolipidemic effect of ursolic acid (50 mg/kg) in rats and Wang *et al*.[35] demonstrated the hypolipidemic effect of ursolic acid (25 mg/kg) in rabbits. In male rats, ursolic acid also showed anxiolytic effect and this effect was comparable to diazepam[36]. Betahistine showed anxiogenic effect but no effect on locomotion. Imaizumi *et al.*[37] also reported the same, but in their study betahistine showed a significant reduction in locomotion and this effect may be mediated through $H_1$ receptor. Both ursolic acid and betahistine reduced the ELT, suggesting that short-term hippocampus-dependent spatial memory is improved[38]. Ursolic acid 40 mg/kg increased the muscular coordination and this may be due to inhibition of muscle breakdown *via* genetic signalling[15].

At the end of the study, olanzapine showed increased levels of glucose, liver enzymes and cellular metabolites/ nitrogenous end products and this was supported by histopathological reports. In absolute and relative weight of organs, betahistine showed a reduction in the weight of lungs and kidneys. The reason for weight reduction is unknown. Olanzapine is known to cause hyperglycaemia and hypertriglyceridemia, and the same was reported in many studies[39-41]. The animal treated with ursolic acid also showed hepatoprotective effect and the same was reported by Shukla *et al*.[42]. Ursolic acid normalizes the bile flow in a dose-dependent manner and anticholestatic property as it increases the bile flow, bile acids, and bile salts, which possibly due to induction of bile salt-dependent fraction or repair of bile duct[43].

Olanzapine treated rats were observed to have increased in their aminotransferase (ALT, AST) level

compared to other groups. Olanzapine has been reported to be causing the most transient, asymptomatic increase in hepatic enzyme among all these atypical antipsychotics. The pathogenesis of olanzapine-associated hepatotoxicity is still not well known and is mostly a transient phenomenon[44]. In histopathology, olanzapine, betahistine, and ursolic acid-treated groups showed mild degeneration of hepatocytes and this may be due to olanzapine-induced metabolic changes including dyslipidemia[45]. Rats treated with ursolic acid showed a reduction in aminotransferase level, maybe due to its antioxidant properties[46]. During the study, the animal administered with olanzapine + betahistine and olanzapine + ursolic acid 10 mg/kg (Gr. V) showed animal mortality and the reason for the death of an animal from each of these groups is not clear. But the olanzapine has two times higher risk on the incidence of sudden cardiac or sudden unexpected death compared to nonusers in humans[47].

## Conclusion

Chronic administration of olanzapine increased body weight and altered the metabolic pattern of experimental animals whereas ursolic acid at 40 mg/kg significantly resisted the olanzapine-induced metabolic changes which include hyperglycemia, hyperlipidemia, and weight gain. These effects of ursolic acid at 40 mg/kg were comparable with that of betahistine 10 mg/kg. From the results, it is inferred that ursolic acid at 40 mg/kg inhibits olanzapine-induced metabolic changes and, this effect may be due to its action on insulin signaling, protein kinase B, adiposity, blood glucose level and/or oxidative stress.

## Conflict of interest

The authors declare no conflict of interests.

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