ORIGINAL ARTICLE

Olanzapine induced biochemical and histopathological changes after its chronic administration in rats

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Abstract *Objective:* Olanzapine is a second generation antipsychotic acting mainly as a dopamine D_2 and serotonine 5-HT_2 receptors antagonist prescribed in the treatment of schizophrenia and various other psychiatric illnesses. Even though olanzapine is widely used in psychiatry, its effects on the architecture of pancreas, liver and kidneys are little known. The histology of pancreas especially has never been studied. For these reasons, the current study was designed to elucidate the toxic effects of chronic administration of olanzapine on pancreas, liver and kidneys and the enzymes released by these tissues in an escalating dose manner. *Methods:* Fourteen male rats were divided into two groups equally, the olanzapine group and the controls. Olanzapine was administered in a dose of 5 mg/kg/d for the first eight weeks, 10 mg/kg/d for next four weeks and 15 mg/kg/d through the last two week period of 14 weeks experiment. The controls received acidified saline only. Both the groups received restricted diet (20 g/12 h). The body weight and level of random blood sugar (RBS) were measured on a weekly basis. The levels of lipase, amylase, alanine transaminase (ALT) and aspartate transaminase (AST) were determined terminally. At the end of the experiment, the tissues were dissected out for histopathological evaluation. *Results:* Significant loss in body weight, change in the level of random blood sugar ($^{**}P < 0.05$, $^{***}P < 0.001$) and significant rise in amylase and lipase levels ($^{*}P < 0.05$, $^{***}P < 0.001$) were observed. However, the same treatment has shown no significant change in the levels of alanine and aspartate transaminases

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($P > 0.05$). The pancreas has shown derangement of beta cells and fibrotic growth. A mild to moderate focal increase in glomerular cellularity, cellular proliferation and glomerular capsules with negligible basement membranes were observed in the kidneys. No changes were observed in the architecture of the liver. *Conclusion:* The findings of this study indicated that the incidence of adverse effects associated with olanzapine could be prevented/alleviated/delayed by allowing restricted diet.

1. Introduction

Olanzapine is an atypical antipsychotic which is commonly used in the treatment of schizophrenia and bipolar disorders. Numerous studies have found that metabolic syndrome is highly prevalent in schizophrenic patients on olanzapine therapy than in general population (Houseknecht et al., 2006). These metabolic events observed in the patients include weight gain, dyslipidemia, insulin resistance and hyperglycemia mirroring impaired glucose metabolism (Houseknecht et al., 2006). Olanzapine has been reported to have strong association with new onset diabetes (Liebzeit et al., 2001). Weight gain is one of the risk factors of diabetes, except a few reports where diabetes did occur without any weight gain in patients subjected to olanzapine. Discontinuation of antipsychotic drugs (APDs) has been observed to decrease the plasma glucose level (Kohen et al., 2008; Koller et al., 2003a; Waage et al., 2004; Wirshing et al., 1998). It has also been shown in animal models that the APDs cause hyperphagia, which results in weight gain followed by hyperglycemia (Cope et al., 2005). Olanzapine-induced acute pancreatitis was first of all reported in 2000 (Doucette et al., 2000). Food and Drug Administration's MedWatch Surveillance System and published reports have indicated one hundred and ninety-two patients with pancreatitis receiving one or the other antipsychotic, with 33% due to olanzapine only (Koller et al., 2003b; Waage et al., 2004). To the best of our knowledge, no study on olanzapine-induced pancreatitis and olanzapine induced changes in the architecture of pancreas has been reported so far in rats. Pae and his colleagues have reported elevation of liver enzymes in rats treated with olanzapine (2 and 4 mg/kg/d, i.p) for 6 weeks but the general architecture of liver has been reported unaffected (Pae et al., 2005), concluding it as damage to hepatocytes at cellular level.

Nephrotoxicity with chronic administration (6 weeks) of olanzapine (0.5 and 2.5 mg/kg/d) has been reported with postulation that it was due to oxidative stress (Mustafa et al., 2012). Dietary and pharmacological interventions have been recommended to lessen the APDs-associated alarming events with the suggestion to study the impact of APDs on body weight by gradually escalating the dose (Gohlke et al., 2012). The underlying mechanism of olanzapine-induced pathological changes has been an issue of great concern among the scientific community, but no or insufficient data exist about the correlation of biochemical and histopathological changes. The current study was therefore designed to assess the effects of chronic (4 months) oral administration of gradually increasing doses of olanzapine on blood glucose, ALT, AST, amylase, lipase and body weight in correlation with its effects on the architecture of pancreas, liver and kidneys while allowing restricted diet.

2. Methods and materials

2.1. Animals

Sprague–dawley male rats were bred in animal house, department of pharmacy, University of Peshawar. Total 14 animals, weighing 160–300 g, were housed in plastic cages at a controlled climate (19–23 °C). The rats were maintained on 12 h reversed light–dark cycle (light on between 21:00 and 9:00 h), started 2 weeks before the start of dosing, and were handled gently and carefully so that the animals were acclimatized for experimental conditions. The animals were then equally stratified into 2 groups, the olanzapine treated group and the controls, each comprising of 7 animals. They were having free access to water and measured quantity of food (20 g/12 h/animal) (Pouzet et al., 2003). All the procedures were approved from the Departmental Ethical Committee (Reference number, 02/EC-14/Pharm) and were in strict accordance with Animals Scientific Procedures Act, 1986, UK.

2.2. Procedures

2.2.1. Drugs treatment

The animals were kept fasted for 24 h before first dose was administered. Olanzapine was formulated by dissolving it in small quantity of 0.1 N acetic acid followed by volume make-up with normal saline in a ratio of 1–100 (Pouzet et al., 2003). Olanzapine was used in once daily doses of 5 mg/kg/d (Pouzet et al., 2003) for first 8 weeks, 10 mg/kg/d for next 4 weeks (Andreasen et al., 1995; Pouzet et al., 2003) and 15 mg/kg/d for last 2 weeks (Angelucci et al., 2005). Controls were administered acidified normal saline in a volume based on body weight (not greater than 5 ml/kg/d) (Pouzet et al., 2003). Both olanzapine and saline freshly prepared were administered orally by oral gavage method during the same period daily (07:00–09:00) (Pouzet et al., 2003; Terry et al., 2003).

2.2.2. Random blood sugar

Random Blood Sugar was measured before the administration of the first dose and then weekly, using Abbott Glucometer.

2.2.3. Enzymology

Enzymes including amylase, lipase, ALT and AST were determined terminally. For enzymes determination, animals were anesthetized with ketamine (100 mg/kg) (Ali et al., 2013),

blood was collected by cardiac puncture, centrifuged, and the serum collected was stored at 4–8 °C which was then analyzed for the levels of enzymes using spectrophotometer (Unico 1100RS).

2.2.4. Body weight measurement

The animals were weighed before the administration of first dose as well as weekly before the administration of next newly calculated dose. The percent weight gain was calculated by using the following formula.

$$\text{Percent gain in body weight} = \frac{100 \times \text{weight (g) on respective week}}{\text{Weight (g) on zero weeks}} \tag{1}$$

2.3. Histological study

2.3.1. Hemotoxylin and Eosin (H & E) staining

The animals were sacrificed, selected tissues isolated, washed with phosphate buffered saline (PBS) and fixed in neutral buffered formalin (NBF) for not less than 6 h. The fixed samples of tissues were sliced into small pieces of 3–5 mm and embedded separately in paraffin blocks. These blocks were sectioned using microtome (SLEE MAINZ, CUT 5062). Sections of different sizes (5 μm and/or 10 μm) were taken and stained using H & E staining technique. The stained slides were analyzed under a light microscope (LABOMED LX400) equipped with camera (iVu 3100). The images obtained were labeled, saved and interpreted for any drug induced changes (Ali et al., 2013).

2.4. Statistical analysis

Data were expressed as mean ± standard error of the mean (SEM). The significance of difference among mean values was analyzed either by one way ANOVA followed by Bonferroni's multiple comparison test or by unpaired t-test using GraphPad Prism 5 (Graph Pad Software Inc. San Diego CA, USA).
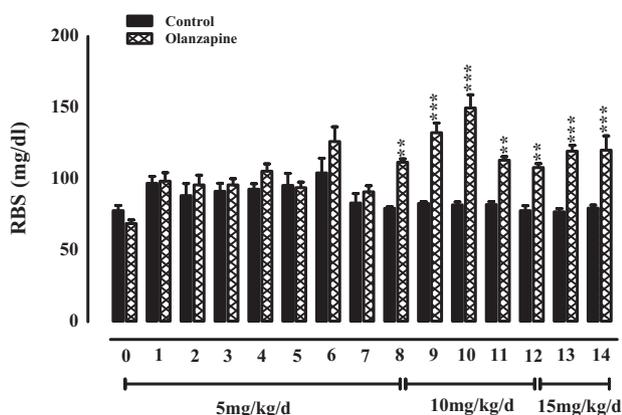
3. Results

3.1. Random blood sugar

Random blood sugar (RBS) was determined on a weekly basis. Olanzapine showed a significant rise in blood sugar in the 8th to 14th weeks of the experiment ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$, Fig. A.1).

3.2. Pancreatic Function Tests (PFTs) and Liver Function Tests (LFTs)

Olanzapine treatment showed a significant rise in amylase ($^*P < 0.05$) and lipase levels ($^{***}P < 0.001$). Olanzapine showed no significant change in ALT and AST levels ($P > 0.05$) (Fig. A.2).

3.3. Percent weight gain

Olanzapine treatment showed significant percent weight gain in controls as compared to olanzapine treated group in the 5th week of first 8 weeks treatment ($^*P < 0.05$, $^{**}P < 0.01$, $^{**}P < 0.001$) (5 mg/kg/d), in the next 4 weeks treatment ($^{***}P < 0.001$) (10 mg/kg/d) and in the last 2 weeks treatment (15 mg/kg/d) ($^{***}P < 0.001$). Nonetheless, the animals treated with saline showed normal growth pattern (Fig. A.3).

3.4. Histological results

3.4.1. Effect on pancreas

As depicted in Fig. A.4, chronic treatment with olanzapine induced pathological changes in pancreas, including derangement of beta cells and fibrotic growth. The exocrine portion remained unaffected.
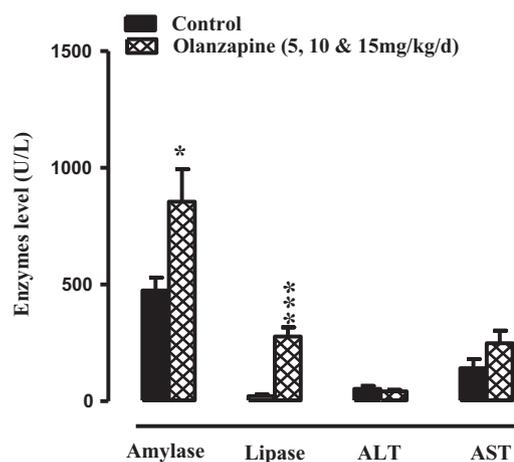
Figure A.2   Effect of olanzapine on enzymes: Olanzapine was administered orally to male rats ($n = 7$) at a dose of 5 mg/kg/d for the first eight weeks, which was increased to 10 mg/kg/d in the next four weeks and then to 15 mg/kg/d in the last two weeks, which showed a significant increase in amylase (U/L) ($^*P < 0.05$) and lipase ($^{***}P < 0.001$) levels (U/L) compared to controls treated with acidified N/S. The same treatment did not cause any change in the levels of alanine and aspartate transaminases ($P > 0.05$). (unpaired t-test).

Figure A.1   Effect of olanzapine on random blood sugar (RBS): Olanzapine was orally administered to male rats ($n = 7$) at a dose of 5 mg/kg/d for the first eight weeks, which was increased to 10 mg/kg/d in the next four weeks and then to 15 mg/kg/d in the last two weeks. (ANOVA followed by Bonferroni's Multiple Comparison Test) ($^{**}P < 0.01$, $^{***}P < 0.001$).
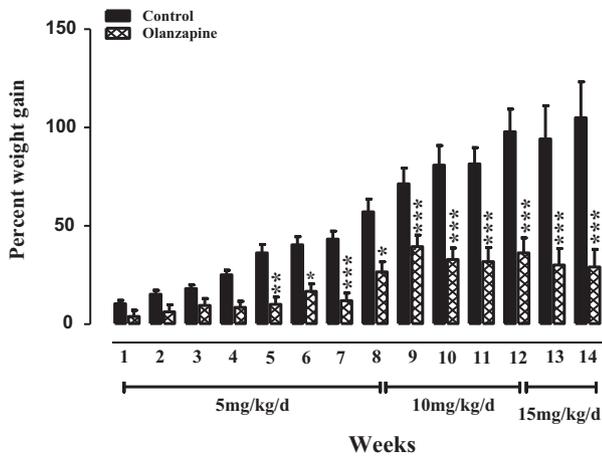
Figure A.3 Effect of olanzapine on body weight: Olanzapine was orally administered to male rats ($n = 7$) at a dose of 5 mg/kg/d for the first eight weeks, which was increased to 10 mg/kg/d in the next four weeks and then to 15 mg/kg/d in the last two weeks. The significant percent weight gain was observed in controls as compared to olanzapine treated group. (ANOVA followed by Bonferroni's Multiple Comparison Test). ($^*P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$).

3.4.2. Effect on liver

Our study revealed normal parenchyma of liver showing no prominent drug induced changes. The center of lobules revealed central vein with thin walls and hepatic portal triad composed of a single portal vein, an artery and the bile duct at the periphery. Hepatic plates composed of hepatocytes were arranged in an interanastomosing network of single cell thick trabeculae separated by vascular channels/sinusoids forming hexagonal structures with central vein (Fig. A.5).

3.4.3. Effect on kidneys

As depicted in Fig. A.6, a mild to moderate focal increase in glomerular cellularity and cellular proliferation were observed in the kidneys. A few glomerular capsules with negligible basement membranes could be identified.

4. Discussion

The previous studies show inconsistent findings about weight gain and hyperglycemia (Baptista et al., 1987). Weight gain has been very serious issue frequently and controversially reported with olanzapine and other APDs (Arjonaa et al., 2004; Gohlke et al., 2012; Wirshing et al., 1998). In this study, significant weight loss was observed with olanzapine that is not in line with most of the studies reported in humans where such treatment has shown weight gain, however, there are also reports that male rats have shown no or little change in weight with an oral dose of 5 mg/kg/d (Pouzet et al., 2003). Several mechanisms of olanzapine have been postulated, including its antagonistic effect on D_2, 5-HT_{2C}, α_1, α_2, H_1 and M3 receptors, but still no decisive conclusion has been elucidated about the mechanisms of its toxicities. Some authors have correlated the weight gain with leptin and/or ghrelin, which cause hyperphagia (Arjonaa et al., 2004; Baptista et al., 1987; Cope et al., 2005; Sentissi Othman et al., 2008). In addition to weight loss
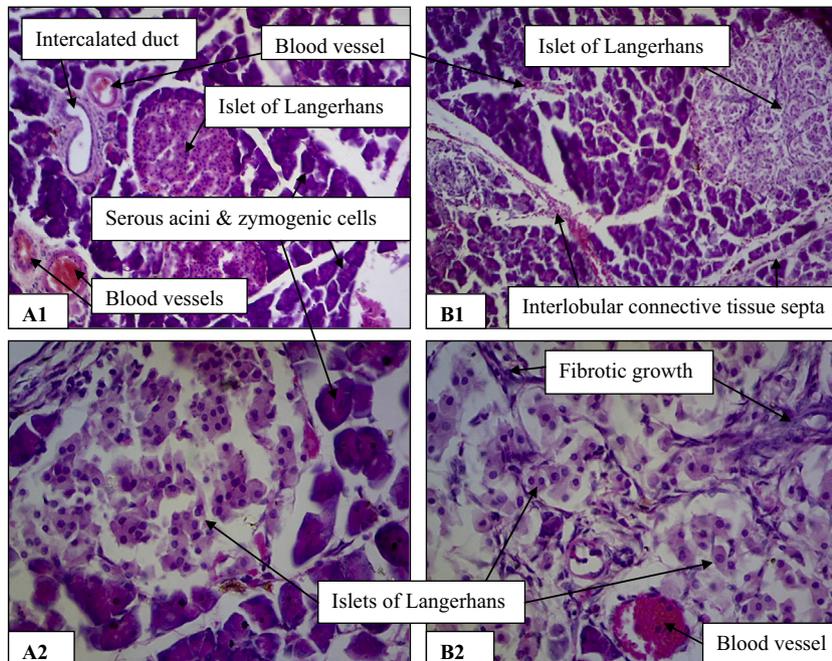
Figure A.4 Olanzapine induced histopathological changes in the pancreas (H & E; A1, B1; 100× and A2, B2; 400× original magnifications): Photomicrographs of a section (5 μm) of pancreas from a rat treated with normal saline (A1 and A2) or olanzapine (5 mg/kg/d for the first eight weeks, 10 mg/kg/d in next four weeks and 15 mg/kg/d in the last two weeks). The saline treated pancreas showed the islet of Langerhans interspersed among the pancreatic exocrine acini. The exocrine component consisted of serous acini and zymogenic cells. The olanzapine treated pancreas showed nodular appearance of islet of Langerhans due to fibrotic growth with derangement of beta cells.
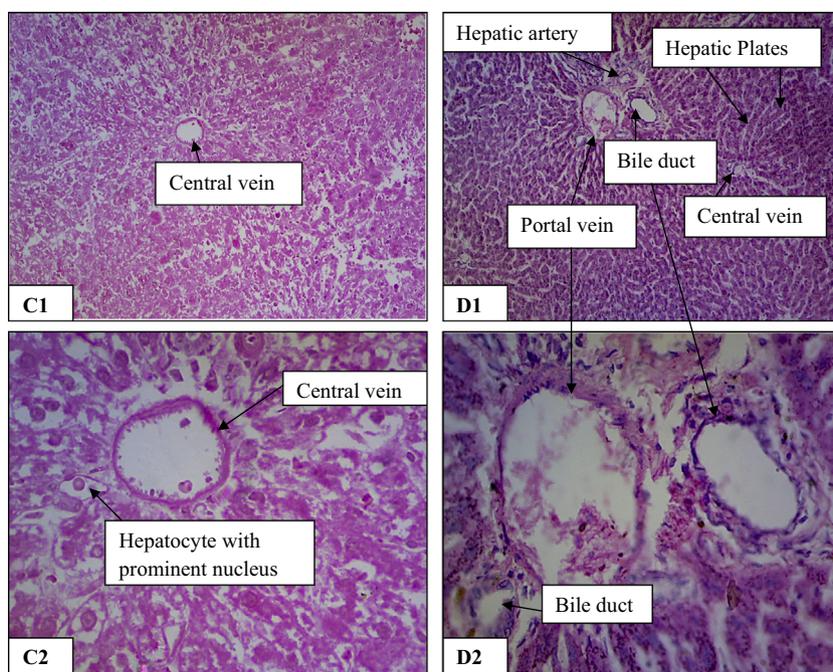
Figure A.5 Olanzapine induced histopathological changes in the liver (H & E; C1, D1; 100× and C2, D2; 400× original magnifications): Photomicrographs of a transverse section (5 μm) of liver of rats treated for 14 weeks with normal saline (C1 & C2) or olanzapine (5 mg/kg/d for the first eight weeks, 10 mg/kg/d in next four weeks and 15 mg/kg/d in the last two weeks) (D1 & D2), showing normal cellular architecture. The center of lobules revealed center thin walled vein with portal triad containing single portal vein, an artery and the bile duct at the periphery. Hepatocytes were arranged in an interanastomosing network of single cell thick trabeculae separated by vascular channels/sinusoids forming hexagonal structures with central vein and radiating hepatocytes trabeculae.
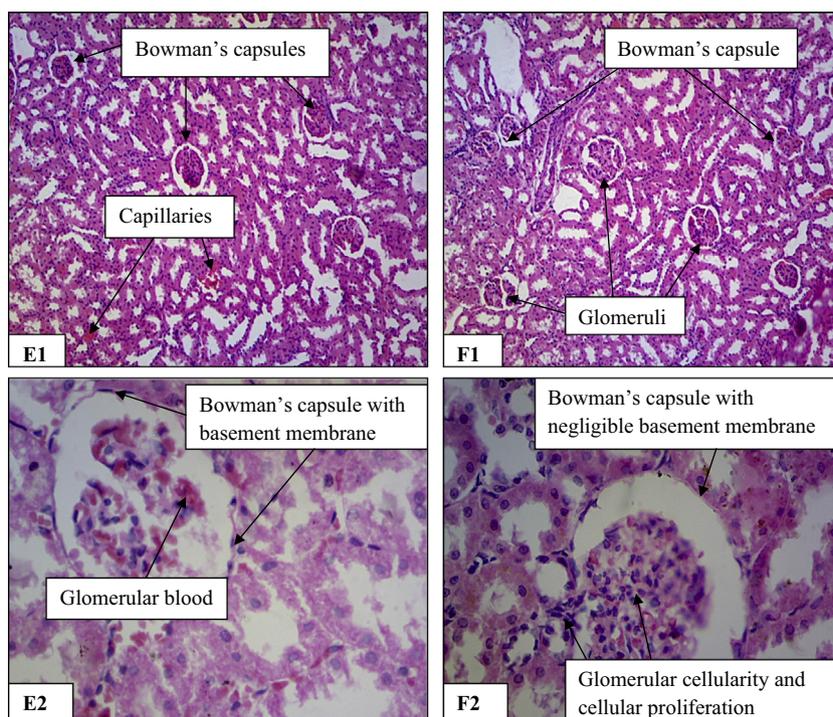
Figure A.6 Olanzapine induced histopathological changes in the kidneys (H & E; E1, F1; 100× and E2, F2; 400× original magnifications): Photomicrographs of a section (5 μm) of the kidneys of rats treated with normal saline (E1 & E2) or olanzapine (5 mg/kg/d for the first eight weeks, 10 mg/kg/d in next four weeks and 15 mg/kg/d in the last two weeks) (F1 & F2), showing mild to moderate focal increase in glomerular cellularity and cellular proliferation. A few glomerular capsules with negligible basement membranes could be identified.

in this study, olanzapine showed significant effects on blood glucose level, but much later in the 8th week of the study, when treated orally for an extended period of time, still the rise in blood sugar is much lower than the previous studies in animal models having free access to food.

Until now the scientists have tried to elucidate the mechanism of toxicities associated with olanzapine, but no or very little attention has been paid to correlate the alterations in biochemistry with histology of the affected organs. There are rare reports on hepatotoxicity and nephrotoxicity but no study on pancreatic toxicity has been found. Therefore the novelty of this study is the simultaneous correlation of both biochemistry and histology of pancreas and liver. Likewise, the drug caused rise in blood sugar, rise in amylase and lipase as well as an insult to the architecture of the pancreas, including derangement of beta cells and fibrotic growth in the islet of Langerhans. The damage could be more severe if exposure to drug would continue and free access to food allowed. The damage could be due to blockade of muscarinic M_3 receptors since studies have discovered that the binding affinity of second generation antipsychotics to the M_3 receptors is a predictor of diabetes risk. Second generation antipsychotics-induced insulin dysregulation may be partly due to blockade of central and peripheral muscarinic M_3 receptors, causing an initial disruption to insulin secretion and glucose homeostasis that can progressively lead to insulin resistance and diabetes during chronic treatment (Weston-Green et al., 2013). Olanzapine alters the density of M_3 receptors in discrete nuclei of the hypothalamus and caudal brainstem, the regions that regulate glucose homeostasis and insulin secretion through vagal innervations of the pancreas (Weston-Green et al., 2013). The pancreas is innervated by adrenergic and cholinergic neurons, which have an indirect effect on the release of insulin through sympathetic and parasympathetic vagal stimulation. Time-sensitive response of insulin to APDs has been reported that is decrease in its level after acute exposure (14 days) to an APD while increase in its level after chronic exposure accompanied with hyperglycemia, the later, reflects insulin resistance (Chiu et al., 2010; Weston-Green et al., 2013). A cohort of Taiwanese schizophrenics was examined for the effects of olanzapine on glucose-stimulated insulin response which showed hypoinsulinemia in the first two weeks of olanzapine treatment, returned to baseline levels in the next two weeks and then hyperinsulinemia in the next four weeks of treatment (Chiu et al., 2010; Weston-Green et al., 2013). In addition to previous hypothesis, hyperglycemia could be due to direct damage to the pancreas especially the beta cells of islet of Langerhans and oxidative stress that is reflected by derangement of beta cells, fibrotic growth in the pancreas and rise in amylase and lipase levels in our study.

There exists a single study on olanzapine-induced hepatotoxicity supplemented with the histopathological findings that reported no damage to the general architecture of liver except to hepatocytes at cellular level, using doses of 2 and 4 mg/kg/d i.p for six weeks (Odaci et al., 2009). Liver enzymes elevation has been, however, reported extensively, but asymptomatic and returned to normal when the drug was discontinued (Gonzalez-Heydrich et al., 2003; Ozcanli et al., 2006). On the other hand, Gomez and colleagues studied more than 2000 patients treated with olanzapine who exhibited no symptoms of jaundice or clinical hepatitis at six month follow-up (Gomez et al., 2000).

Similarly there are rare reports on olanzapine-induced nephrotoxicity as well but with low dose (2.5 mg/kg/d) injected intraperitoneally for 6 weeks, however the dose of 0.5 mg/kg/d has been reported safe (Mustafa et al., 2012). Furthermore, we found mild to moderate focal increase in glomerular cellularity, cellular proliferation and Bowman's capsules with negligible basement membranes.

The reasons of inconsistent findings with the previous studies could be due to various factors including (1): Male gender (Pouzet et al., 2003), (2): Access to restricted diet (Gohlke et al., 2012), (3): Chronic exposure to high dose of olanzapine that could have developed resistance, (4): Once daily dosing though with shorter half life of 2½ h (Van der Zwaal et al., 2008), (5): Possible degradation of olanzapine in solution before administration (Van der Zwaal et al., 2008), and (6): Four month study is still shorter to induce pathological changes in liver, since 2000 patients after six month follow-up showed no hepatotoxicity (Gomez et al., 2000). Out of all of the above postulations, the importance of restricted diet cannot be ignored. Our results indicate that one may possibly protect patients from the untoward effects by devising diet with a calculated amount of calories during the course of treatment with APDs. The effect of calories intake has also been established by Fountain and his colleagues in healthy human volunteers (Fountaine et al., 2010).

We consider it as an advancement in the major issues associated with olanzapine which might be lessened with dietary interventions, as predicted previously. Dietary interventions would be more fruitful than anti-obesity drugs due to the concerns of untoward effects. Not only weight gain, all other alarming events such as dyslipidemia, cardiovascular risks and hyperglycemia might be managed if restricted and balanced diet is provided to the patients on antipsychotics. The other very important aspects regarding weight in schizophrenics could be hypothesized that it is not the APDs that cause weight gain and metabolic syndrome rather schizophrenia that causes weight loss and metabolic abnormalities. The drug might be hitting the same pathway(s) that would have been reversed by the schizophrenia. This could be observed in clinics/hospitals that the majority of the schizophrenics are underweight and unable to control pulse rate and blood pressure which start improving with the use of APDs. Our study has shown some positive expectations from the postulation of dietary interventions in patients on antipsychotic therapy. But still the risk of toxicity may not be ignored, as evident from pathological changes in the architecture of kidneys and pancreas. Since olanzapine has been the mainstay of treatment strategies for schizophrenics and patients with other affective disorders, it must be used with caution and regular monitoring of body weight, renal functions, blood glucose, lipid profile, amylase and/or lipase shall be exercised, so that the risk to the patients could be minimized. We suggest switching to other safe antipsychotics when the patient's previous condition is improved with olanzapine. It may be restricted for use in reactive and non-responding cases only and then switched to safer drug when the previous symptoms are alleviated.

5. Conclusion

The study helps us in concluding that olanzapine is comparatively safer to the liver, but may be toxic to pancreas and

kidneys. Furthermore, we found that the toxicities associated with olanzapine might be lessened if the diet provided is intervened, since our dietary intervention has delayed the already reported events for weeks. Based on this study and the published reports, it is suggested to monitor body weight, blood glucose, LFTs, RFTs, lipid profile and amylase and/or lipase in patients undergoing therapy with olanzapine.

6. Limitations

LFTs, amylase, lipase and histological examinations were done terminally which could not be correlated with the doses being used. Further, histopathological findings of kidneys were not supplemented with RFTs.

Acknowledgment

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