

LONG TERM EFFECTS OF OLANZAPINE CUMULATIVE DOSES ON FAT TISSUE. AN EXPERIMENTAL MODEL IN RATS

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Abstract

Olanzapine, a second generation antipsychotic drug, is frequently used to treat schizophrenia due to several advantages that it has over classical antipsychotics in terms of side effects. However, the occurrence of certain side effects adverse reactions with regards to long term administration is mentioned in the medical literature. The aim of our study was to investigate the effects of various doses of olanzapine, given for 9 weeks, on body weight and to evaluate the drug's pharmacotoxicological tropism related mostly to fat and liver tissues in Wistar rats. Olanzapine was administered by gavage in doses of 0.5, 2.0, 4.0 and 6.0 mg/kgbw, respectively, to 70 adult Wistar rats with body weights ranging from 260 to 330 grams, and other two groups serving as controls received only vehicle solution, without any active ingredient. During the experiment, the animals were weighed weekly, and in the end they were euthanized, when samples from their fatty and liver tissues were taken for subsequent histological analyses. The results indicated a relative weight gain of the animals from all treated groups compared to their initial state, the observed changes being gender and dose related, excepting the 6 mg/kgbw group where toxicity signs were noticed during the study. Histological analyses of adipose and liver tissues revealed obvious and significant irreversible inflammatory alterations after 9 weeks of treatment, correlated with the total cumulated olanzapine dose and with the gender of the animals. High doses of olanzapine caused toxic reactions in the second part of the study.

Rezumat

Olanzapina face parte din categoria antipsihoticelor moderne, fiind frecvent utilizată în tratamentul schizofreniei, având o serie de avantaje privind efectele secundare față de antipsihoticelor clasice. Cu toate acestea, date din literatura de specialitate semnalează apariția unor efecte secundare /reacții adverse la administrarea acestora pe termen lung. Obiectivul studiului nostru a fost de a urmări efectele olanzapinei, administrată la șobolani albi Wistar, în doze diferite timp de 9 săptămâni, asupra curbei ponderale și a evaluării tropismului farmacotoxicologic cu predilecție asupra țesutului adipos și hepatic. S-au utilizat 70 de animale, șobolani albi Wistar, adulți cu greutatea cuprinsă între 260 g și 330 g cărora li s-a administrat prin gavaj, olanzapină în doze de 0,5, 2,0, 4,0 și 6,0 mg/kgcorp, și două loturi martor, care nu a primit medicație, doar vehiculul. Pe parcursul experimentului, animalele au fost cântărite săptămânal, iar la sfârșit animalele au fost sacrificate și s-a prelevat țesutul adipos și ficatul care ulterior au fost analizate histologic. Rezultatele arată o creștere în greutate a animalelor față de greutatea inițială la toate loturile tratate, modificările fiind dependente de sex și de doza administrată, cu excepția lotului care a primit olanzapină în doză de 6 mg/kgcorp, unde s-au înregistrat semne de toxicitate pe parcursul studiului. Analiza histologică a depozitelor adipoase, respectiv a ficatului arată modificări semnificative, cu fenomene inflamatorii evidente, corelate cu doza totală cumulativă, cu caracter ireversibil.

Keywords: olanzapine, adipose tissue, rats, weight gain

Introduction

Schizophrenia affects more than 1% of the adult population worldwide, being a chronic disease requiring lifelong treatment [12].

Modern antipsychotics such as olanzapine, clozapine and risperidone, which preferentially block the central nervous system (CNS) 5-HT₂ receptors in a much greater extent than they block

dopamine D₂ (10:1) receptors, have exhibited significantly diminished side effects compared to classical neuroleptics (lack of extrapyramidal reactions, reduced incidence of hyperprolactinaemia). However, the use of these new drugs may lead to the occurrence of metabolic syndrome, characterized by weight gain and metabolic disturbances, the phenomenon being most noticeable in the case of

clozapine and olanzapine treatment [8, 14, 18]. Transcriptional changes in peripheral tissues, which contribute to the metabolic state of the body, were also described [22]. Olanzapine is one of the most prescribed antipsychotic drugs, due to its higher efficacy in treating both positive and negative symptoms of schizophrenia, and its efficacy in treating bipolar disorder, autism, mania and depression [5, 16, 19]. Although there is a low probability that olanzapine treatment causes extrapyramidal side effects and prolactin levels are not significantly influenced, there are major concerns regarding metabolic disturbances arising from its long-term use. There has been a few research studies performed so far to evaluate the potentially dose-dependent toxic effects of the drug. Since the data published in the literature with regards to olanzapine's adverse effects has not yet

been adequately clarified and is still controversial, the objective of the present study was set to investigate the effects of several dosing regimens over 9 weeks in Wistar rats and to analyse the changes in growth, body weight and adipose tissue.

Materials and Methods

There were used 7 groups of Wistar rats (males and females, respectively as described in Table I for the exact gender distribution) treated at fixed time intervals every morning by oral gavage with olanzapine doses of 0.5, 2.0, 4.0 and 6.0 mg/kgbw respectively, for a period of nine weeks. Two groups of animals only received vehicle solution and served as absolute controls. The individual body weight of the study animals ranged between 260 and 330 grams, at the beginning of the experiment.

Table I
Experimental protocol

Group No.	No. of animals / gender	Medication and Dose
1.	10 Wistar rats - male	0.5 mg/kg bw- olanzapine
2.	10 Wistar rats - female	0.5mg/kg bw- olanzapine
3.	10 Wistar rats - female	2.0 mg/kg bw- olanzapine
4.	10 Wistar rats - female	4.0 mg/kg bw- olanzapine
5.	10 Wistar rats - female	6.0 mg/kg bw- olanzapine
6.	10 Wistar rats - male	Vehicle – without active substance
7.	10 Wistar rats - female	Vehicle – without active substance

1 mL of 1.0 % Tween 80 aqueous solution was used to dissolve the olanzapine dose administered daily to each animal.

The animals were housed at the Târgu Mureş University of Medicine and Pharmacy's bio-base throughout the study, they were fed *ad libitum* with standard diet and water, an optimal ambient temperature of $22 \pm 2^\circ\text{C}$ and 30 - 60% humidity was constantly provided. The animals were administered the active substance or vehicle by oral gavage on a daily basis, and were monitored for possible side effects of the drug at 1, 2 and 4 hours after the treatment. Animals were weighed weekly to assess changes in body weight. All the experimental procedures were performed according to the international guidelines regarding research on laboratory animals, in accordance with the guidelines of EU Directive 2010/63 and with the approval of the Institutional Review Board - Ethics Committee of the University of Medicine and Pharmacy in Târgu Mureş.

After 9 weeks of treatment, the animals were euthanized with sodium pentobarbital i.v. and death was confirmed by the onset of *rigor mortis*.

Hepatic and adipose tissue samples were collected (adipose tissue was sampled from the abdominal and subcutaneous regions), were weighed for each animal in the group and subsequently processed and analysed histologically.

Histological analysis

Hepatic tissue and adipose tissue fragments collected from subcutaneous and abdominal depots

were fixed in buffered 4% paraformaldehyde at 4°C for 48 hours and then processed through dehydration and clarification for paraffin embedding. Paraffin blocks were cut at 4 μm thickness, dewaxed, rehydrated and coloured with H&E (haematoxylin and eosin) stain. Slides were observed and registered with a Nikon Eclipse microscope coupled with a digital camera. Images were finally processed using the Microsoft Office Picture Manager.

Statistical analysis

Results are expressed as mean \pm standard deviation (SD). If passed Kolmogorov-Smirnov normality test, continuous variables were compared using One-way ANOVA tests or repeated measures One-way ANOVA, as the experimental design imposed. *Post hoc* comparisons were performed using Bonferroni test. Unpaired or paired Student t-test were used to compare means of two groups, as appropriate. Non-normally distributed data were evaluated using Kruskal-Wallis or Friedman test, with *post hoc* Dunn's Multiple Comparison Test, Mann-Whitney U test and Wilcoxon matched pairs test, respectively. Statistical significance limit was set to a p value < 0.05 .

Results and Discussion

Considering the weight gain, the greatest difference compared to baseline weight was recorded from the

first week of treatment in groups of animals treated with a dose of 2.0 mg/kgbw olanzapine, while a significant increase was recorded in the female group treated with 4.0 mg/kgbw olanzapine. In groups treated with 0.5 mg/kgbw olanzapine, the initial weight loss was noticed for both genders, then a slight increase was noticed in males and higher weight gained the females.

During the study, a change in behaviour was observed in the treated groups, with decreased spontaneous motility, state of indifference and sluggishness. The group of female rats receiving 6.0 mg/kgbw showed weight gain during the first 4 weeks, then there was a stagnation or even a slight decrease in body weight in some of the animals compared to the initial state, which has not been

previously mentioned in the literature. Signs of toxicity (wheezing, coughing and choking) were also recorded for the 6 mg/kgbw dose. These toxicity symptoms might be correlated with hypercoagulability, which has been observed for this group. It is also possible that this effect was not related to the administered drug, assuming events that could otherwise occur during the study – virosis or inflammatory processes.

Analysing the body weight variation during the treatment period revealed a marked increase in olanzapine treated groups. The change in body weight was even more evident in female rats, where a statistically significant increase was reached in a shorter time interval (Figures 1, 2 and 3).

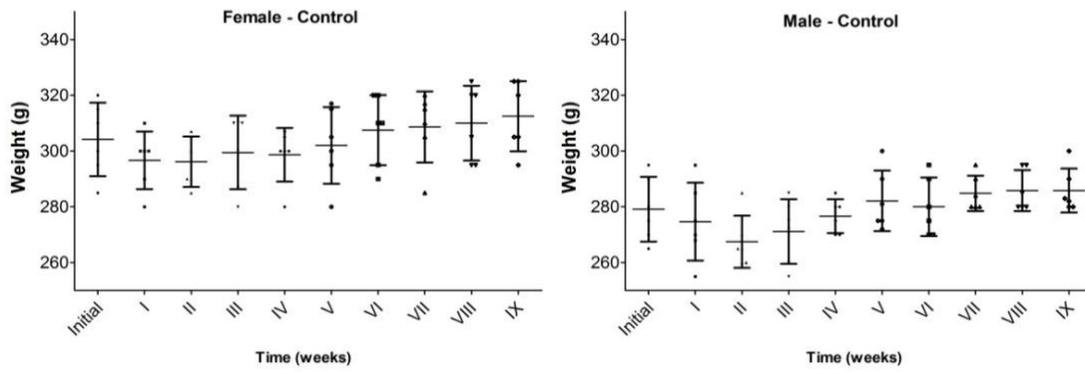


Figure 1.

Body weight variations of rats in the control groups during nine weeks treatment period. Data are expressed as means \pm SD. Arrows indicate the earliest observed statistically significant difference compared to initial state in each group ($p < 0.05$).

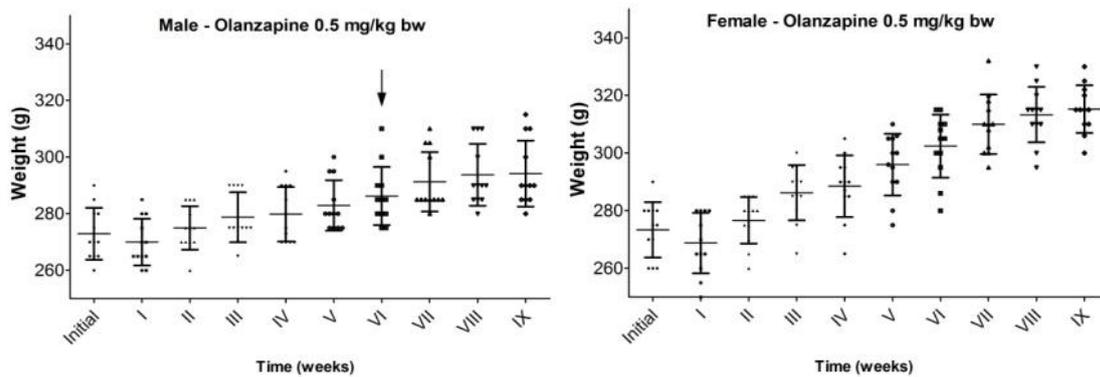


Figure 2.

Body weight variations of rats treated with 0.5 mg/kgbw olanzapine during the nine weeks treatment period. Data are expressed as means \pm SD. Arrows indicate the earliest observed statistically significant difference compared to initial state in each group ($p < 0.05$).

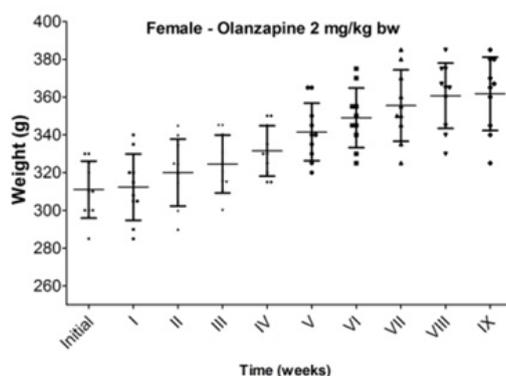


Figure 3.

Body weight variations of rats treated with 2.0 mg/kgbw olanzapine during the nine weeks treatment period. Data are expressed as mean \pm SD. Arrows indicate the earliest observed statistically significant difference compared to the initial state in each group ($p < 0.05$).

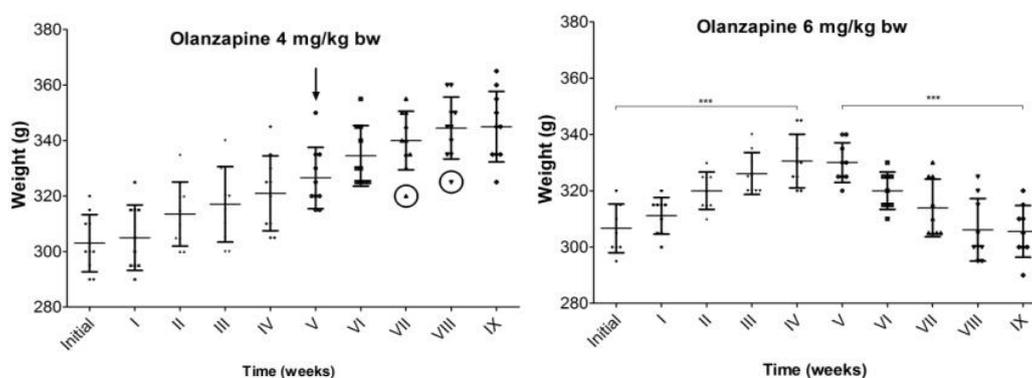


Figure 4.

Body weight variations of rats treated with 4.0 and 6.0 mg/kgbw olanzapine respectively during the nine weeks treatment period. Data are expressed as means \pm SD. Arrows indicate the earliest observed statistically significant difference compared to initial state in each group ($p < 0.05$). *** - $p < 0.001$, statistically significant difference obtained with Dunn's Multiple Comparison test. Circles indicate the reduction of body weight compared to the previous week.

At the highest dose of 6.0 mg/kgbw, olanzapine produced a significant initial increase in body weight after the first four weeks of treatment ($p < 0.001$), but a significant reduction of this parameter was observed in

the last four weeks of the treatment period ($p < 0.001$; Figure 4).

A significant increase in body weight was observed within each group, with regards to weekly recorded differences (Table II).

Table II

Body weight changes during the treatment period

Gender	Groups	Mean difference between final and initial weight (g)	Friedman statistic	p
Male	Control	6.60	32.97	< 0.001*
	Olanzapine 0.5 mg/kgbw	21.30	97.49	< 0.0001
Female	Control	8.30	31.84	< 0.001*
	Olanzapine 0.5 mg/kgbw	42.00	103.00	< 0.0001
	Olanzapine 2.0 mg/kgbw	50.70	87.08	< 0.0001
	Olanzapine 4.0 mg/kgbw	42.00	87.19	< 0.0001
	Olanzapine 6.0 mg/kgbw	-1.11	61.83	< 0.0001

* No statistically significant difference was observed between initial and final weight of the animals, but a statistically significant fluctuation was observed using Dunn's Multiple Comparison test during the treatment period.

The increase was most obvious in the females that received 2.0 mg/kgbw and 4.0 mg/kgbw of olanzapine, respectively.

Abdominal fat was noticed in large quantities even in the groups treated with a dose of 0.5 mg/kgbw olanzapine, as compared to the control groups (Table III).

The largest amount of fat was found in the female group treated with a dose of 2.0 mg of olanzapine/kgbw, where the difference was significantly higher compared to the control group,

and the lowest amount of fat was found in the male group treated with a dose of 0.5 mg of olanzapine/kgbw, the differences being very small, compared to the control group.

Table III

The amount of abdominal fat in the study groups

	Total abdominal fat (g)	p values
Control – Female	8.8 ± 1.1	
Control – Male	7.9 ± 0.9	
Olanzapine 0.5 mg/kgbw – Female	10.9 ± 0.9	< 0.001
Olanzapine 0.5 mg/kgbw – Male	8.1 ± 1.1	> 0.05
Olanzapine 2.0 mg/kgbw – Female	14.8 ± 1.2	< 0.001
Olanzapine 4.0 mg/kgbw – Female	14.1 ± 0.9	< 0.001
Olanzapine 6.0 mg/kgbw – Female	12.1 ± 1.3	< 0.001

Histological examination of the adipose depots

The adipose depots collected from controls and rats treated with various doses of olanzapine were sectioned and stained for histological examination. Usual staining with haematoxylin-eosin (H&E) revealed differences between controls and treated animals with respect to adipose tissue histology. H&E staining of adipose tissue sections collected from control rats revealed the characteristic features for each kind of adipose depot assessed (Figures 5 and 6).

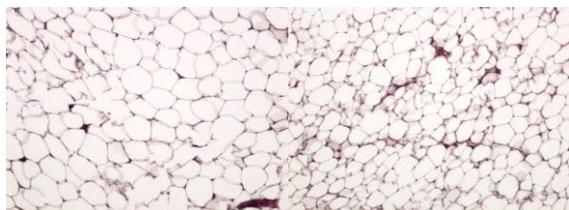


Figure 5.

Visceral adipose depot from a control rat: intraperitoneal depot with unilocular polyhedral or oval adipocytes, relatively constant in size (a, x100); well vascularized perirenal depot with adipocytes of various sizes, but smaller than those from the peritoneal WAT (b, x100)



Figure 6.

Subcutaneous adipose depot from a control rat. Unilocular polyhedral adipocytes almost identical in size, with an abundant stromal vascular fraction (a, x100, b, x400)

Samples from control rats revealed few macrophages, cells that may be involved in the

tissue remodelling process, needed for adapting to various amounts of fat to be stored or used.

Analysis of adipose tissue samples collected from rats treated with olanzapine revealed morphological changes compared to those from control rats. Both the visceral (Figure 7 a - e) and peripheral adipose depots (Figure 7f) presented signs of adipocyte hypertrophy and hyperplasia.

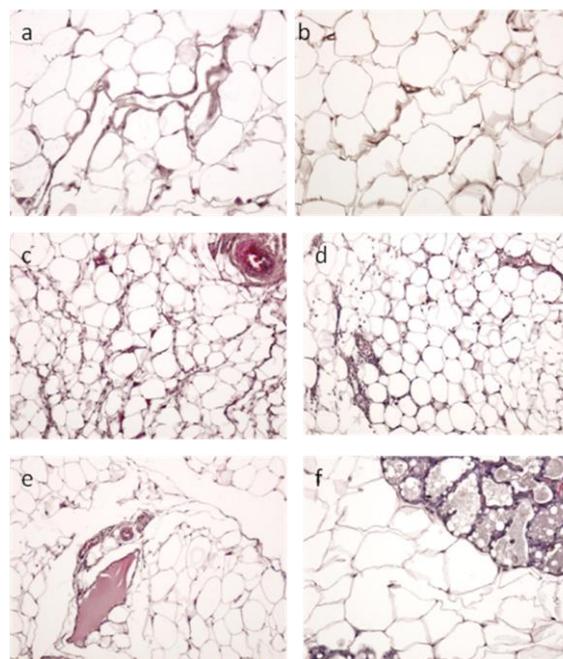


Figure 7.

Adipose depots from rats treated with olanzapine (intraperitoneal - a. 0.5 mg; b. 2.0 mg; c. 4.0 mg; d. 6.0 mg; perirenal - e. 6.0 mg; subcutaneous - f. 6.0 mg) revealed aspects of adipocyte hypertrophy and hyperplasia and the presence of numerous immune cells (H&E, x200, x400, x100, x100, x100, x400)

Cell contour presented many anfractuositities, with irregular or interrupted cell membranes, and increased numbers of immune cells in the visceral pads (Figure 8).

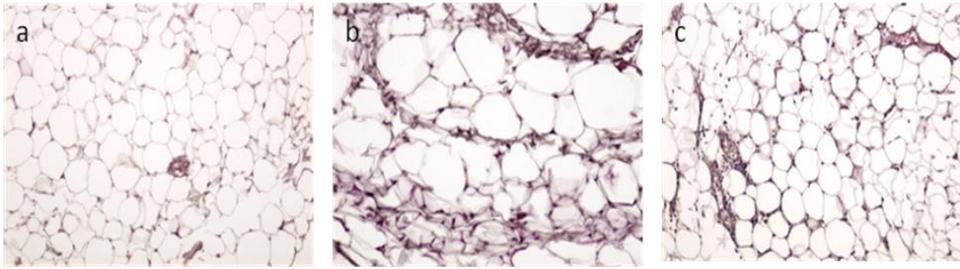


Figure 8.

Immune cells infiltration of the adipose depots collected from olanzapine-treated rats increased as the dose raised from 2.0 mg (a), to 4.0 mg (b) and 6.0 mg (c) (H&E, x100, x200, x100)

High power microscope objective lenses revealed that these cells – monocytes, lymphocytes, polymorphonuclear cells, plasma cells and macrophages – were more abundant as the olanzapine dose increased (Figures 9). High incidence of immune cells was accompanied by an increase of the fibrillary elements in the non-adipocyte fraction. In this experiment, we observed that macrophages infiltrated in the stromal vascular fraction form “crown-like” structures located around the adipocytes (Figure 10).

Even if morphological and histological changes were also observed in the subcutaneous adipose pads, they were less infiltrated with macrophages than the visceral depots (Figure 11).

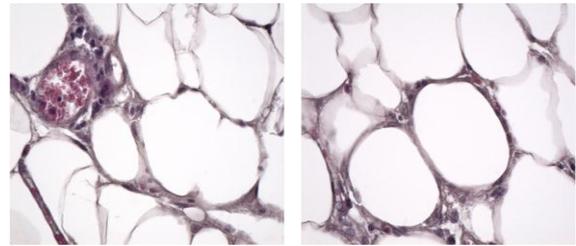


Figure 10.

Adipose depots from rats treated with 6.0 mg of olanzapine. Stromal vascular fraction infiltrated with “crown-like” aggregates of macrophages surrounding the adipocytes and many dilated blood vessels (H&E x400)

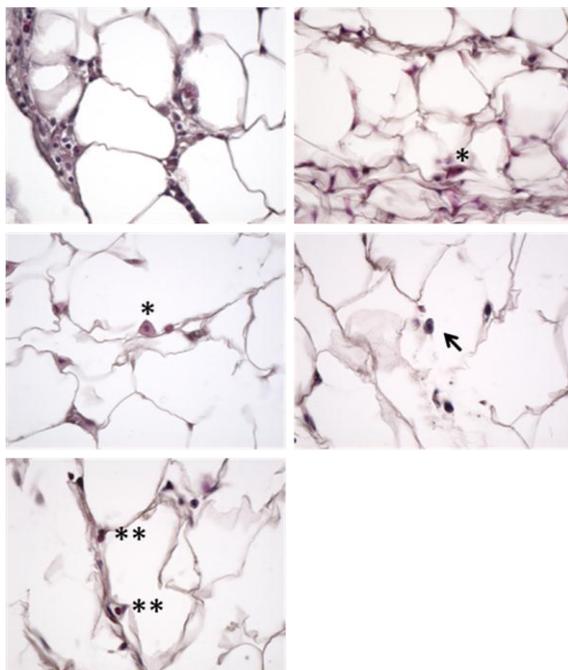


Figure 9.

Various types of immune cells infiltrated in the visceral adipose pads of olanzapine treated rats: many lymphocytes, plasma cells (*), macrophages (<), polymorphonuclear cells (**) (H&E, x400)

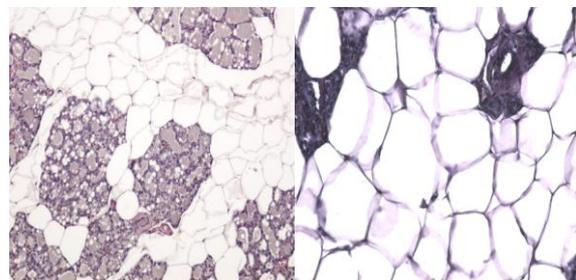


Figure 11.

Subcutaneous adipose pads collected from rats treated with 6.0 mg of olanzapine/kgbw. Diminished inflammatory infiltration compared to visceral depots. Lobules of mammary gland reveal that the adipose tissue was collected from the anterior part (H&E x200, x400)

Assessment of hepatic toxicity was done using liver tissue samples that were prepared for histological examination and stained with haematoxylin eosin. Hepatocyte morphology, centrilobular veins and portal fields were observed for signs of inflammation, cellular regeneration or reparative processes (cicatrisation, including fibrosis) (Figures 12-17).

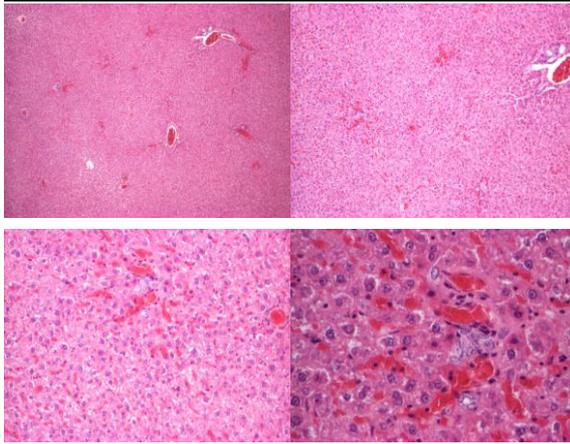


Figure 12.

Olanzapine 2.0 mg/kgbw - Microscopic description:
Mild vascular stasis – dilated sinusoid capillaries,
filled with red blood cells.

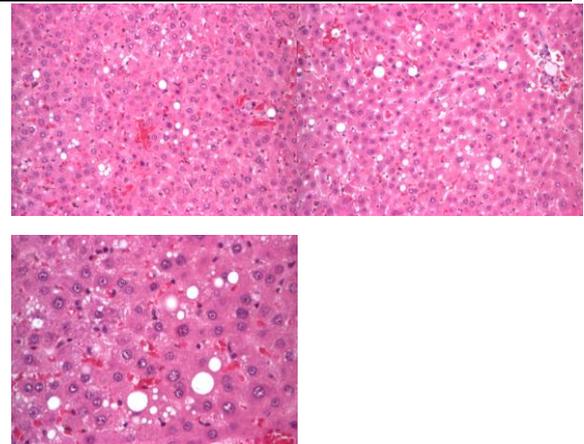


Figure 15.

Control samples for olanzapine – normal
hepatocytes

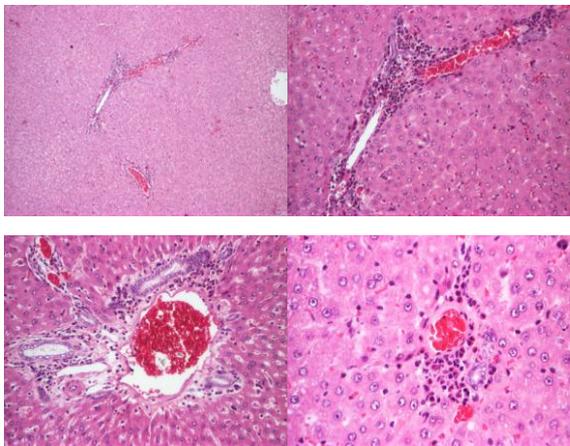


Figure 13.

Olanzapine 4.0 mg/kgbw - Microscopic description:
mild chronic inflammatory infiltrate in the portal fields,
with polymorphic character: lymphocytes, segmented
neutrophil granulocytes and numerous eosinophils.

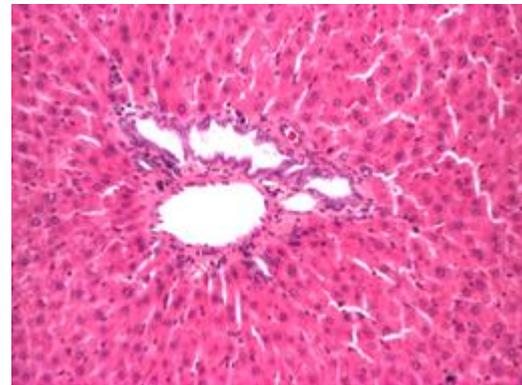


Figure 16.

Portal field – control sample

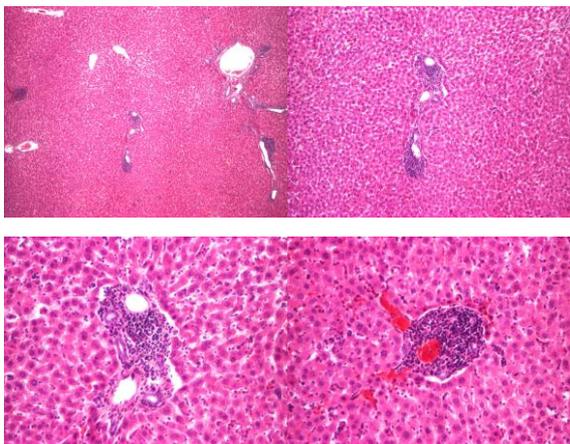


Figure 14.

Olanzapine 6.0 mg/kgbw - Microscopic
description: abundant chronic inflammatory infiltrate
in the portal fields; unorganized micro and macro
vacuolar intra hepatocyte adipose dystrophy.

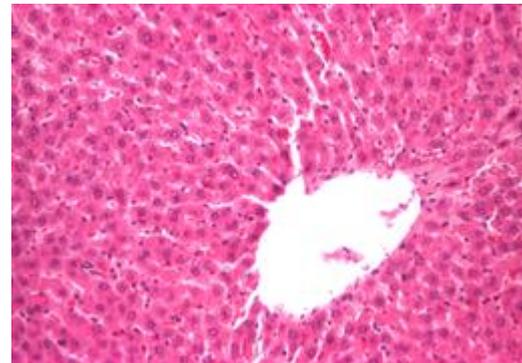


Figure 17.

Centrilobular vein – control sample

Our research results have shown body weight gain, which was most obvious in the group of females treated with a dose of 2.0 mg olanzapine/kgbw. An increase in body weight was also recorded with the dose of 4.0 mg olanzapine/kgbw, similar to the increase noticed with the 0.5 mg/kgbw dose. Weight gain was very slight in the group of males treated with 0.5 mg of olanzapine/kgbw, but yet statistically significant compared to the control group. These results are in accordance with

previously reported observations that recorded certain side effects of antipsychotics, such as gender dependent weight gain [4, 11, 15].

A change in behaviour was observed in the treated groups during the 9 weeks of the study, with an obvious decrease of spontaneous motility, state of indifference and slowness, similar results being reported by other research groups [23]. It appears that chronically administered olanzapine produces hyperphagia associated with body weight gain and increased fat accumulation [6, 21].

The amount of adipose tissue collected from the treated groups was significantly increased compared to the control group. Accumulation of fat in the animals that have not greatly increased in weight implies significant metabolic changes. However, molecular mechanisms have not yet been elucidated [7]. The underlying mechanism might be olanzapine's blockade on two types of receptors, 5-HT_{2C} and H₁, both regulating the food intake. Moreover, a central role of leptin was also proposed for hyperphagia, as hypothalamic insensitivity to this hormone was also observed with olanzapine administration [14].

Difficulty in breathing, choking associated with cough might be related to hypercoagulability, which was revealed when blood samples were taken for further investigations [2, 9, 10, 20]. The adverse effect might as well not be related to the administered drug, assuming events of different kinds – viral infections or inflammatory processes.

After performing the histological analysis we found changes in colour and a “fish-egg” – like granular appearance of the adipose tissues in olanzapine-treated rats, such morphological alterations being also reported by Tan *et al.* in their studies [17]. These changes were observed in both genders, even if the weight gain was limited in male rats.

Subcutaneous and visceral adipose tissues were composed mainly by unilocular polyhedral or oval white adipocytes, which varied in size with nutritional status and type of depot. Cells from the visceral adipose depots were smaller than those from subcutaneous depots with a decreased capacity of fat storage, prone to cell necrosis in conditions of cell hypertrophy when the critical cell size is exceeded [1, 3].

We found that olanzapine treatment induced histological changes of both visceral and subcutaneous adipose pads, in both genders. Still, the histological changes were dose-dependent and more pronounced in female rats. The increase of adipocyte number and size was accompanied by an infiltration of immune cells, because adipocyte hypertrophy/hyperplasia generate areas of local micro hypoxia and the necrosis that follows may be a stimulus to regulate macrophage infiltration of the adipose tissue. We also observed an increase of the

non-adipocyte fraction of the adipose tissue, which also seemed to be dose-dependent.

Our findings are generally in accordance with those of Mann *et al.*, who also reported a significant increase of visceral fat in olanzapine-treated rats compared to vehicle treated animals [11]. Studies performed by Tan *et al.* reported the same morphological and histological changes of subcutaneous adipose pads, and they suggest that adipocyte proliferation in olanzapine-treated rats was dose and time dependent [17]. The more important changes of the visceral depots suggest that visceral fat storage could be more sensitive to olanzapine than peripheral accumulation.

Changes of the non-adipocyte fraction could be accompanied by a defective extracellular matrix (ECM) storage that signals inflammatory cell infiltration in a similar manner as collagen VI, the main protein of the adipose tissue's ECM [13].

The hepatic toxicity described in this experimental model has a definite but currently underdiagnosed clinical correspondent, most likely hidden by the use of polymedication in schizophrenic patients. The targeted toxicity of olanzapine might serve as an experimental model for liver dysfunction, useful for the screening of potentially protective agents (Sengupta P *et al.*, 2010) [23]. The mechanism of liver toxicity was revealed by Eftekhari A *et al.*, and seems to involve oxidative stress related phenomena, involving reactive oxygen species, favouring of lipid peroxidation and the depletion of glutathione's reduced state, all depending on the CYP450 mediated biotransformation; secondary hepatic toxicity mechanisms appear to include mitochondrial changes, through cellular lysis and induced apoptosis, and also by massive release of lysosomal origin proteases [7, 24].

Conclusions

We concluded that the study demonstrated an obvious increase in body weight in female Wistar rats treated with a dose of 2.0 mg olanzapine/kgbw, as this group presented the most significant accumulation of fat. With the 4.0 mg/kgbw dose, the increase in body weight was evident but small, as well as the quantity of adipose tissue; toxicity signs were observed with the 6.0 mg/kgbw dose, such changes being somewhat correlated with the alterations observed in the adipose tissue.

This study provided evidence that besides the significant body weight gain, olanzapine treatment also induced various morphological changes in adipose depots. Adipocyte hypertrophy causes local areas of hypoxia in the adipose tissue and the subsequent adipocytes necrosis represents an important stimulus for phagocytosis. The stroma from the visceral pads of olanzapine-treated rats

displayed an increased infiltration of macrophages forming “crown-like” structures around white adipocytes. Liver toxicity manifests gradually, along with the cumulated dose, featuring signs of discreet inflammation that extend up to unorganized micro and macro vacuolar intra hepatocyte adipose dystrophy.

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