



Hepatotoxic changes induced by prenatal administration of olanzapine in swiss albino mice

KEYWORDS

Pregnancy, oral gavage, haemopoietic cell, Teratogenic drugs, Antipsychotic drugs

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ABSTRACT

Introduction : Olanzapine is an atypical antipsychotic drug approved by FDA for the treatment of psychotic disorders. Although atypical antipsychotics have limited evidence for teratological risk, there are reports of altered fetal growth, both increased and decreased, with maternal atypical antipsychotic use. But its effect on fetal liver is yet to be ascertained.

Materials and methods : pregnant albino mice was given olanzapine in the dose of 0.2 mg/kg and amount of distilled water during the same period. On day 19th the mice was sacrificed and fetuses were taken out. The liver of fetuses were dissected, formalin fixed, processed and stained with H&E for histological study.

Results : On gross examination liver of 2mg/kg group showed marked reduction in size of liver. On histological examination in the treated group there is decreased density of haemopoietic cells as well as hepatoblasts. The progenitor cells and hepatoblasts shows degeneration with pyknotic nuclei, karyolysis and karyorrhexis.

Conclusion: olanzapine causes hepatotoxicity in fetus if given to mother during pregnancy and should be used with caution in pregnancy.

Introduction

Women of childbearing age appear to be particularly susceptible to the exacerbation of existing mental illness and the development of new mental illness. Indeed, it has been estimated that over 500,000 pregnancies annually are complicated by psychiatric illness that either precedes pregnancy or arises during pregnancy[1]. Untreated psychiatric disease during pregnancy is associated with increased risks for the mother (including self-harm/suicide, self-neglect, and reduced compliance with prenatal and postnatal care) and risks for the child (including impaired fetal development, infanticide, and impaired mother-child bonding)[2,3]. Historically, antipsychotics have been extensively and effectively used for the treatment of schizophrenia and bipolar disorder, and more recently they are becoming part of the treatment of depression[4]. Conventional antipsychotics (also known as typical or first generation antipsychotics) which were more commonly used to treat these conditions caused a significant decrease in fertility[5]. However, the newer atypical antipsychotics do not have this side-effect.

As a consequence, the number of women taking antipsychotics, who are becoming pregnant, is on the rise. Indeed, appointments at a Mother risk Program Clinic related to the use of antipsychotic medications increased 170% between 1989 and 2001; a rise which was for the most part attributable to an increased use of second-generation or atypical antipsychotics[6]. The vast majority of women who use antipsychotics during pregnancy do so because of ongoing illness. In fact, only in cases of where the first schizophrenic episodes are reported in pregnancy or there is a risk of puerperal psychosis would the exposure of antipsychotic exposure be restricted to the pregnancy period.

The FDA approved Olanzapine, an antipsychotic drug manufactured by the Eli, Lilly and company, in October 1996, for the treatment of psychotic disorders. It is a thieno-benzodiazepine analog with the chemical name of

2-methyl-4-(4-methyl-1-piperazinyl)-10-thieno (2,3-b) (1,5) benzodiazepine. Olanzapine is a yellow crystalline solid and practically insoluble in water. Its structure is shown in Figure 1.

Behavioral pharmacological in vivo studies show that olanzapine is an antagonist of dopamine, serotonin, and acetylcholine [7,8]. This receptor profile parallels that of Clozapine[7,9].

Animal studies have shown rapid placental transfer of olanzapine and it even crosses human placenta. Olanzapine has been found to be genotoxic in humans as it exerts a dose related cytotoxic effect, causes metabolic disruption and mitochondrial DNA depletion on human cells in vitro. It has been classified as class C drug by FDA which means that it has been found to be safe in animals but studies in human beings are inconclusive.

Use of olanzapine during pregnancy is a dilemma faced by the physician as the safety profile of this drug has not yet been established. So, in the present study we intend to observe the toxic effect of olanzapine on liver of the embryo when given to the mother during pregnancy.

Materials And Methods

This study was conducted on 27 swiss albino mice. Prior approval of institutional ethical committee was taken before the start of the present study. For this study swiss albino female mice were taken and kept with male mice for mating overnight in the ratio of 3:1. presence of vaginal plug was considered to be the first day of gestation (GD 0). The pregnant female mice were divided into three groups for the present study.

The first group was designated as control and was given tap water by gavage from day 6 to day 12 of gestation. The other two groups were treated as treated and were

given olanzapine in the dose of 0.2mg/kg (low dose) and 2 mg/kg (high dose) respectively by gavage for the same period. On day 18th of gestation the female mice was sacrificed by cervical dislocation and uterotomy was done to extract the embryos. The liver of the embryos were dissected out and kept in formalin for fixation. For histological study the liver was processed, sections were cut at 8µm and stained with hematoxylin and eosin (H&E).

Results

On gross examination of Liver it was seen that liver of 2mg/kg (high dose) treated group showed marked reduction in size as compared to liver of 0.2mg/kg (low dose) treated group and control. (figure 2,3)

When the weight of liver was taken of various groups it was found that high dose group showed statistically significant reduction of weight as compared to low dose and control group and also the low dose group showed statistically significant reduction of weight as compared to control group. (fig 4)

The microscopic examination of liver of control mice shows haemopoietic cells which are seen in plenty as the liver is a typical haemopoietic organ in fetal life, responsible for development of RBCs and WBCs. interspersed between the haemopoietic cells are the hepatoblasts having vesicular nuclei. Also precursor of platelet, megakaryocytes are also found in the developing fetal liver. The liver sinusoids are still in developing phase and there is still no definite laminar pattern in fetal liver. (fig 5, fig 8)

In low dose 0.2 mg/kg Olanzapine treated liver there is decreased density of haemopoietic cells as well as hepatoblasts. The progenitor cells and hepatoblasts shows degeneration with pyknotic nuclei, karyolysis and karyorrhexis. Due to degeneration of cells the sinusoids appear enlarged. (fig 6, fig 9)

In high dose 2mg/kg Olanzapine treated liver these findings are further amplified. There is intense loss of cells both progenitor as well as hepatoblasts with considerable amount of cellular debris. The cellular population is considerably reduced and there are vast vacuolar spaces seen in the liver. (fig 7, fig 10)

Discussion

Liver is a fast growing parenchymal organ in fetal life where its prime function is haemopoiesis. The cells are in rapid stage of division and differentiation. These fast dividing cells are much more prone to toxic insult in comparison to mature adult cells. The liver of treated mice shows decreased density of haemopoietic cells as well as degenerating hepatoblasts with considerable amount of cellular debris. The severity of the damage increases with dose of drug. This finding is in unison with other studies which found hepatocyte injury, ballooning degeneration and perisinusoidal fibrosis and raised aminotransferases. This could be explained on basis that Olanzapine induces lipid peroxidation which causes increase in Malonyldialdehyde (MDA) and 4-Hydroxynonenal (4-HNE). These oxidative stressors may be responsible for damage to progenitor cells and hepatoblasts[10].

The toxicity of olanzapine results from its deleterious effect on placenta where it acts on the human trophoblast causing alteration in 5HT_{2A} serotonin receptors[11]. These receptors are vital in controlling cellular differentiation, proliferation and migration of cells[12]. Also serotonin results

in altered estrogen biosynthesis which is crucial for successful implantation of blastocyst and regulation of leptin expression[13,14]. Leptin is an important chemical involved in regulation of fetal growth so downregulation of estrogen synthesis and leptin expression may result in IUGR and low birth weight[15]. Indirect role of Dopaminergic receptor (D₂) expression in fetal growth has also been postulated and it may be a key factor in controlling the fetal weight in Olanzapine treated mice. This also explains the reduced size of liver in high dose of olanzapine.

Thus it will be prudent to say that olanzapine causes toxic changes in the fetal liver and should be cautiously prescribed by the physician during pregnancy especially to ladies suffering from any liver disease or predisposed to oxidative stress.

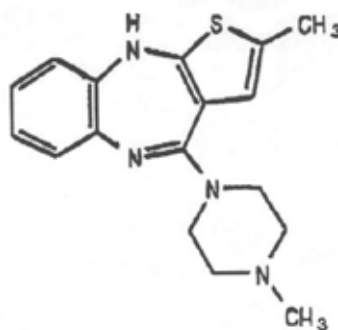


Fig. 1: Chemical structure of Olanzapine

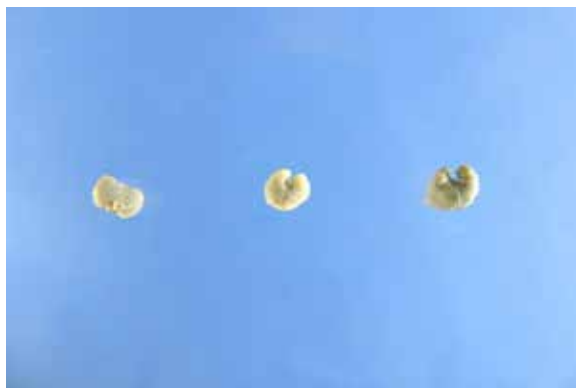


Fig. 2 : gross specimen of liver(superior surface) eliciting reduction in size of liver which is maximum in high dose group

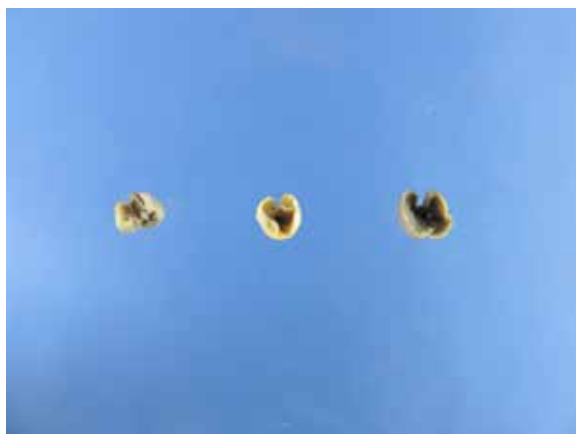


Fig. 3 : gross specimen of liver(inferior surface) eliciting reduction in size of liver which is maximum in high dose

group

Group	Mean±SD	p-value
Group 1	0.272±0.08	0.032 (< 0.05)
Group 2	0.19±0.02	
Group 3	0.145±0.015	

Fig 4. Table indicating the comparative reduction of liver in low dose and high dose group

Group comparison	=	<0.05
Group 1 vs Group 2	=	<0.05
Group 1 vs Group 3	=	<0.05
Group 2 vs Group 3	=	<0.05

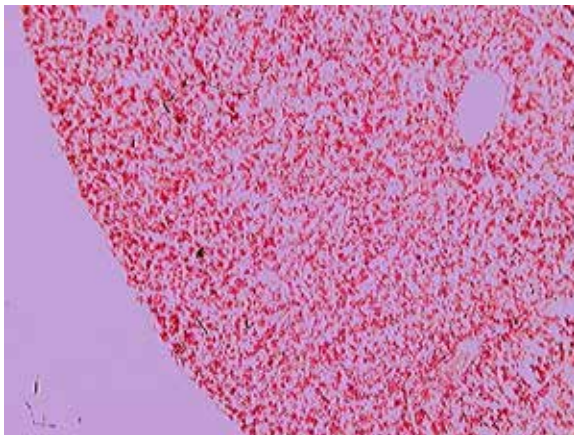


Fig. 5: CONTROL LIVER, H&E, 100X showing central vein , developing sinusoid and non laminar pattern of hepatoblasts

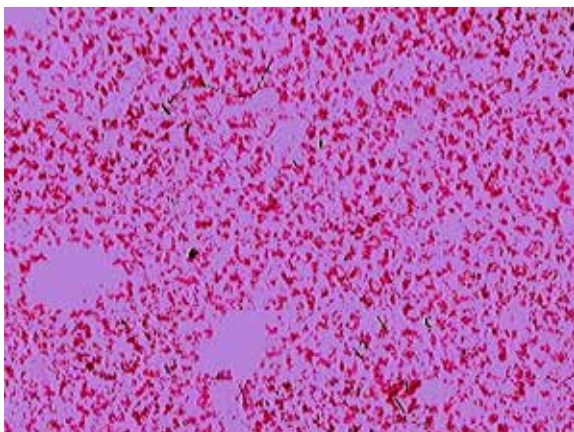


Fig 6 LOW DOSE OLANZAPINE TREATED LIVER, H&E,100X showing low density of hepatoblast ,dilated

sinusoids, disruption around central vein and empty vacuolar spaces depicting degeneration

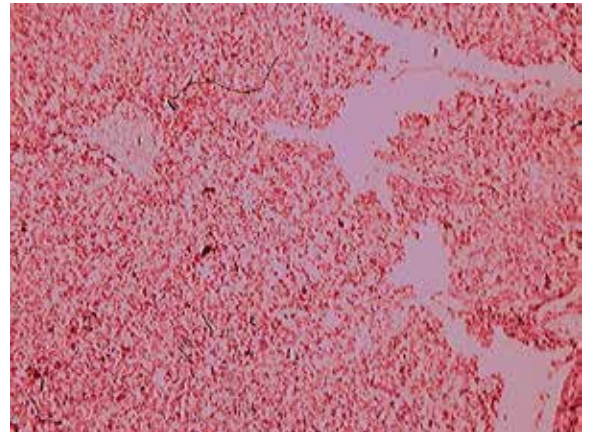


Fig 7 HIGH DOSE OLANZAPINE TREATED LIVER , H&E, 100X showing complete loss of cytoarchitecture and large empty spaces

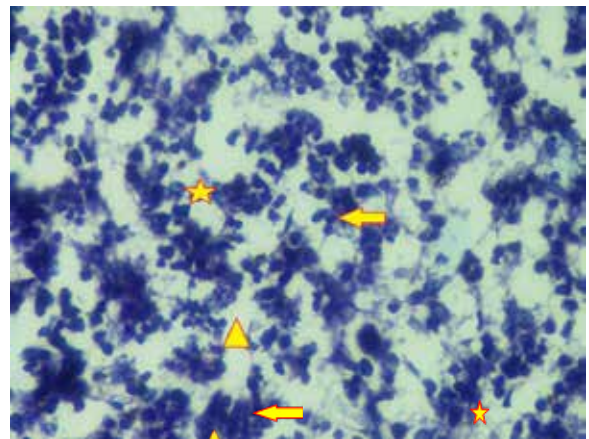


Fig 8 .Photograph of liver of control mice fetus showing haemopoietic cells (☆), hepatoblasts (□) and developing sinusoids (△) (H & E × 400)

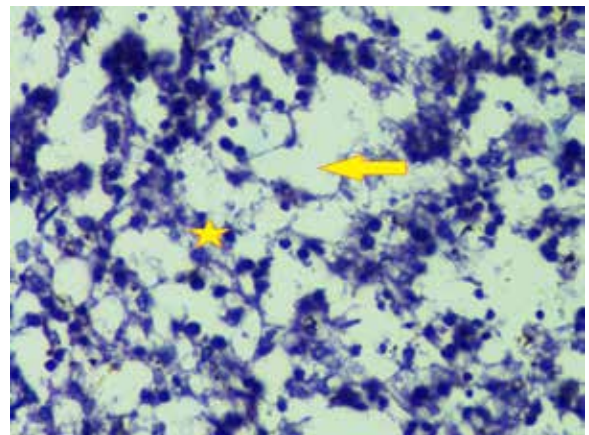


Fig 9. photograph of liver of mice fetus treated with 0.2 mg/kg olanzapine showing degeneration of haemopoietic cells and hepatoblast (☆) resulting in empty vacuolar spaces (→) (H & E × 400)

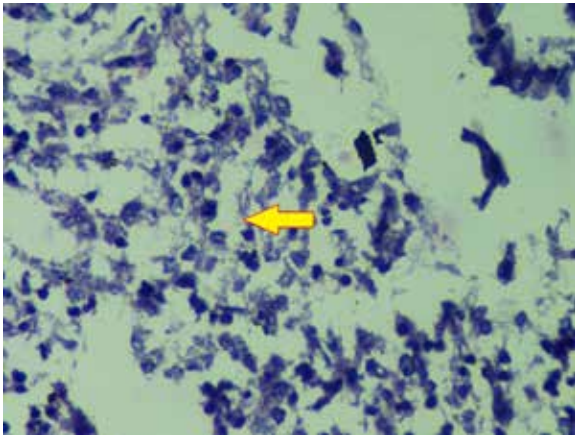



Fig 10 photograph of liver of mice fetus treated with 2mg/kg olanzapine showing necrosis and degeneration of hepatoblasts and complete loss of cytoarchitecture () (H & E × 400)

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