

# Histopathological effect of fluoxetine drug on the brain of pregnant mice and their embryos

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## Abstract

The present study was designed to identify the histopathological effect of fluoxetine (fluoxetine hydrochloride) on the pregnant mice brain and their fetuses as well as recording weight changes at doses of 60 and 80 mg/kg of body weight which were administrated to the pregnant mice from the 7<sup>th</sup> up to the 18<sup>th</sup> day of pregnancy and once a day. The results showed histopathological lesions in the adult pregnant mice brain at the dose of 60 mg/kg. induced massive necrotic area of neurons in the cerebral cortex, necrosis of the molecular layer cells while the dose of 80 mg/kg the changes include vasogenic edema in the sub cortical area, severe degeneration and necrosis of the cortex. The lesions in cerebellum were more severe than recorded in the cerebrum. The results also showed the presence of many lesions in the fetus brain at the dose 60 mg/kg and they were degeneration, necrosis of many neurons in the cerebrum while at the dose of 80 mg/kg. There were increase of degeneration and necrosis in the cortical area of fetus brain. In conclusion, the use of fluoxetine in pregnant mice caused decrease in body weight and Histopathological changes in the brain of both of the mothers and their embryos, thus the use of antidepressant by pregnant woman can be harmful for the mothers and the fetus.

**Keywords:** Histopathology, Fluoxetine, Mice, Brain, Fetus

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## التأثيرات النسجية المرضية لعقار الفلوكستين على دماغ الفئران الحوامل وأجنحتها

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### الخلاصة

صممت الدراسة الحالية لتشخيص تأثير عقار الفلوكستين على دماغ الفئران الحوامل وأجنحتها وكذلك تسجيل التغيرات الوزنية باستخدام الجرعتين 60 و 80 ملغم/كغم من وزن الجسم والتي أعطيت للفئران الحوامل من اليوم السابع إلى اليوم الثامن عشر من الحمل ولمرة واحدة يوميا. أظهرت النتائج آفات نسجية مرضية في دماغ الفأر الحامل عند التركيز 60 ملغم/كغم من وزن الجسم وتمثلت بمنطقة تنخرية واسعة في عصبونات القشرة المخية، فضلا عن نخر الخلايا في الطبقة الجزيئية، أما عند التركيز 80 ملغم/كغم من وزن الجسم فتمثلت الآفات بأدمة وعائية ونخر في خلايا منطقة القشرة الدماغية وكانت الآفات المرضية في الدماغ كانت أكثر شدة مما سجلت في المخيخ. أظهرت النتائج العديد من الآفات في دماغ الأجنة عند التركيز 60 ملغم/كغم من وزن الجسم وتمثلت بتنكس ونخر العصبونات في الدماغ، أما عند التركيز 80 ملغم/كغم من وزن الجسم فقد أظهرت النتائج زيادة التنكس الخلوي ونخر في منطقة القشرة المخية. نستنتج أن استعمال عقار الفلوكستين في فترة الحمل للفئران يتسبب بتغيرات نسجية مرضية في دماغ الأم الحامل وأجنحتها عند تعاطيه بجرعات عالية وفقدان الوزن وعليه نستطيع أن نعد هذا العقار سام إذا استعمل خلال الحمل بجرعات عالية. ونستنتج بهذا أن إعطاء الفلوكستين للفئران يتسبب بتغيرات وزنية تمثلت بانخفاضها فضلا عن ظهور آفات نسجية مرضية في دماغ الفئران الحوامل وأجنحتها وبهذا فإن استعمال الأم الحامل في الإنسان لمضادات الاكتئاب يكون مضر ومؤذي لها ولأجنحتها.

## **Introduction**

Fluoxetine hydrochloride is used to treat a number of psychiatric conditions in humans and behavioral problems in animals (1), it is fluorinated methyl phenoxy derivate of phenylpropylamin, the drug also known by trade name as Prozac, fluoxetine antidepressant of serotonin reuptake inhibitor (SSRI) (2). Serotonin is a neurotransmitter which means it relays signals between cells in the brain (3). The drug ingested by the mouth usually once a day in the morning (4), this drug doesn't only used for the treatment of major and minor depression also used for relieve nausea, vomiting during early pregnancy (5,6). Pregnancy should be carefully evaluated given that it is a period during which woman go through many physical, hormonal and psychic changes which in turn influence the health of both mothers and their fetuses (7). These changes during pregnancy may result in serious emotional problems because fluoxetine and its metabolite norfluoxetine have been shown to cross placental barrier that may cause damage in the major organs of both mother and the fetus such as liver, kidney and other organs, it also may lead to increased extracellular serotonin levels and potentially neurodevelopmental changes in the fetus, it pass the blood brain barrier (8), the fluoxetine transferred from the mother to the fetus through blood plasma so it may spread in to entire the brain of the fetus (9), as well as it may lead to abortion if it used during second and third trimester so that the drug is able to produce a significant histopathological lesions in most vital body organs as well as malformations (10). That means drug can affect the fetus functional development and growth (11). Fluoxetine and its metabolite nor fluoxetine secreted in breast milk and data indicated that these compounds concentration are about 20 - 30 % concurrent maternal plasma drug concentration especially in the rats and these compounds are become at highest level in fetal brain (12). Experimental studies indicated that fluoxetine decrease antioxidant levels, induced oxidative stress and modulating it in different body organs specially the brain that deepened on the taken dose and treatment period (13,14), in the last few years using medications among them fluoxetine increase to treat all emotional and physical changes during pregnancy (15). So that the objective of the present study was to investigate the histopathological changes in the brain of the pregnant mice and their fetuses which treated with fluoxetine and to record the weight changes.

## **Materials and methods**

### **Animals, housing and mating**

The present study was carried on 60 pregnant mice weighting  $30 \pm 3$  gm at age  $60 \pm 4$  days for 3 months. At the time when the experiment started, the animals were

obtained from animal house of Mosul medicine college Mosul University, Mosul - Iraq. All animals housed in clean cages with ad libitum access to water and standard chewable food diet at room temperature  $25 \pm 1$  °C and mice were kept under a natural light - dark cycle (9). For mating mature males were caged at the afternoon for overnight with females in a ratio of one male / two females / cage (16), at the next morning the presence of vaginal plug was considered as the first day of the pregnancy according to the (17), where they still undisturbed until the day 6<sup>th</sup> of gestation the females were transferred from the mating cages and divided into groups. Medication that was used in the present study is fluoxetine 20 mg capsules the production company is Bristol laboratories Ltd, United Kingdom.

### **Experimental design**

The pregnant mice were divided in to three groups and each group consist of 20 pregnant mice (n=20). Group I: control group which administrated orally with 0.2 ml of distilled water from the day 7<sup>th</sup> until the day 18<sup>th</sup> of pregnancy. Group II: which administrated orally with 60mg/ kg from the day 7<sup>th</sup> until the day 18<sup>th</sup> of pregnancy. Group III: which administrated orally with 80mg/ kg of B.W. from the day 7<sup>th</sup> until the day 18<sup>th</sup> of pregnancy. The concentrations 60 m and 80 mg / kg of the fluoxetine were prepared by dissolving each concentration in 5 ml of distilled water (stock solution) and the doses were given depending on the weight of the mice, it was taken from the stock solution according to the mice weight and the dose almost was between 1.5-2 ml which was given to the pregnant mice, the doses were freshly prepared every day, the doses that used in the present study had been chosen depending on the LD<sub>50</sub> of the fluoxetine which is 100mg /kg (18). The pregnant mice were weighted on the 7<sup>th</sup> and 18<sup>th</sup> day of gestation for once by using electronic sensitive balance, all weights were recorded and their rates were calculated for all groups to identify the effect of the drug in the weight between the control group and the treated groups as well as to determine the effect of the dose on the weight of the two groups which treated with concentrations 60 and 80 mg/kg.

### **Histopathological examination**

For Histopathological examination, the animals were sacrificed, dissected after 12 hours from the last dose which was in the 18<sup>th</sup> day of pregnancy, the brain of both mothers and their embryos were isolated and rinsed with normal saline solution, the samples were fixed in formalin 10 % for 48 hours (19), after fixation the brain tissue (brains of the mothers and fetus) were washed with distilled water the samples were treated by routine histological process of paraffin embedded tissue and stained with Delafield's hematoxylin & eosin, sections were mounted with D.P.X.

and examined using optical microscope (20). For photographed a digital camera was used of the type Sony, Cybershot, Japan.

## Results

### Microscopical observations of the brain of adult pregnant mice

Microscopic examination of control cerebrum, which is one of brain parts revealed normal cerebral tissue composed of glial cells and neurons (Figure 1). Sections from the brain of second group were show sever necrotic area of neurons in cerebral cortical area (Figure 2). In third group the sections of brain were show vasogenic edema (Figure 3). Also, there was sever degeneration and necrosis in the brain cortex which revealing extensive destruction of the cerebral tissue (Figure 4).

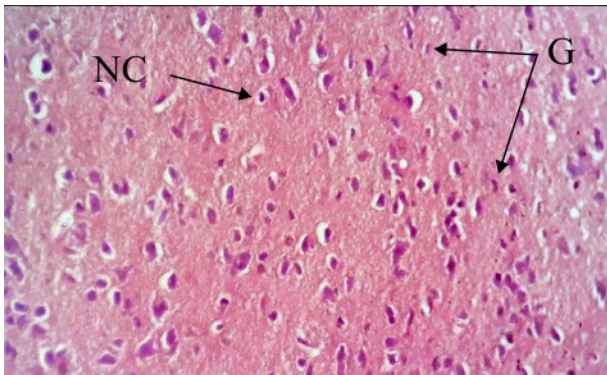


Figure 1: Histological section in a female brain of adult pregnant mice *Mus musculus* (control), composed of glial cells (G) and neurons cells (NC). H&E, 100x.

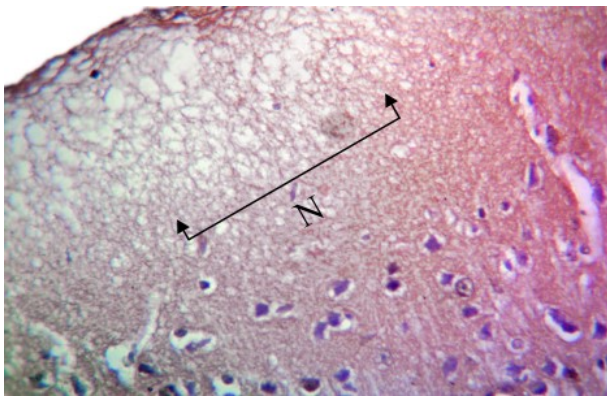


Figure 2: Histological section in a female brain of adult pregnant mice *Mus musculus* treated with fluoxetine drug at a dose of 60 mg/ kg of B.W. from the 7<sup>th</sup> day until the 18<sup>th</sup> day of gestation showing necrotic area (N) in the cerebral cortex. H&E, 400x.

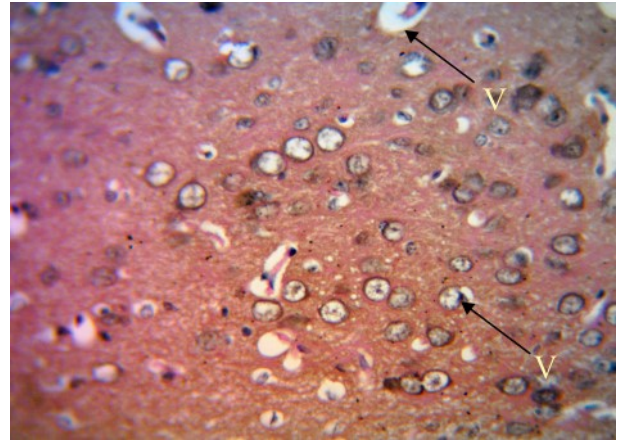


Figure 3: Histological section in a female brain of adult pregnant mice *Mus musculus* treated with fluoxetine drug at a dose of 80 mg/ kg from the 7<sup>th</sup> day until the 18<sup>th</sup> day of gestation showing: vasogenic edema (V). H&E, 400x.

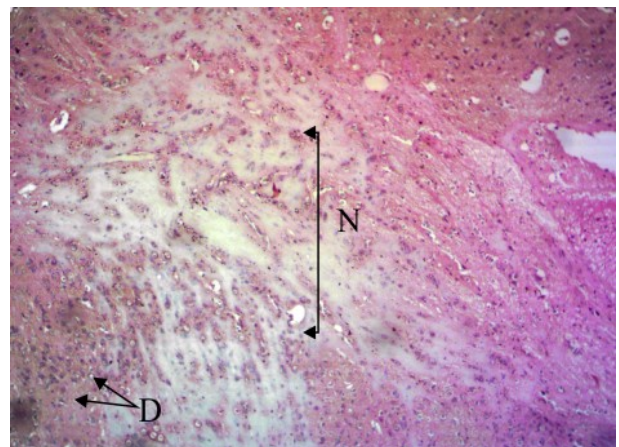


Figure 4: Histological section in a female brain of adult pregnant mice *Mus musculus* treated with fluoxetine drug at a dose of 80 mg/ kg of B.W. from the 7<sup>th</sup> day until the 18<sup>th</sup> day of gestation showing: degeneration (D) and necrosis (N) in the brain cortex. H&E, 400x.

### Weight Changes

Adult pregnant mice weight was measured on the 7<sup>th</sup> and 18<sup>th</sup> day of gestation for control group and both groups which treated with the doses 60 and 80 mg/kg that used in the present study. The results showed that there was a marked decrease in body weight in all treated groups when compared with the control group. As well as there was more decrease in pregnant mice weight which treated with the dose of 80mg/kg of fluoxetine compared to the pregnant mice group which treated with the dose of 60 mg/kg of the same drug (Table 1).



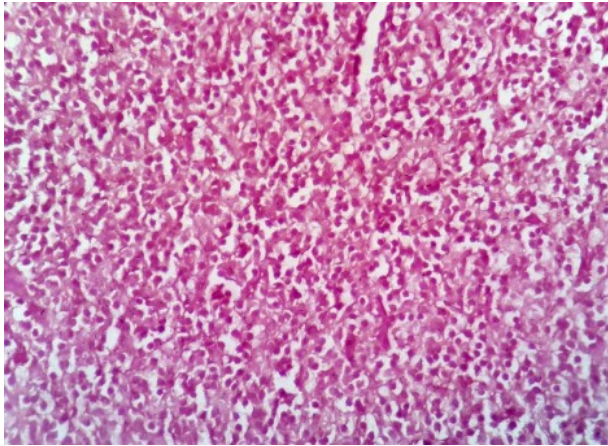


Figure 5: Histological section in a fetus brain of pregnant mice *Mus musculus* (control) showing: primitive normal tissue in the cerebrum. H&E, 400x.

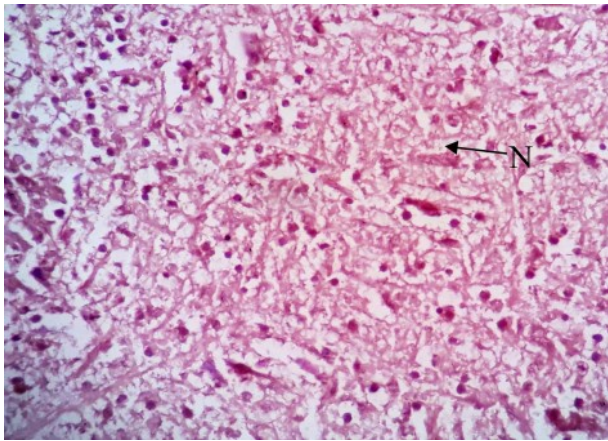


Figure 6: Histological section in a fetus brain of pregnant mice *Mus musculus* treated with fluoxetine drug at a dose of 60 mg/kg from the day 7<sup>th</sup> until the day 18<sup>th</sup> of pregnancy showing: necrosis (N) of many neurons in the cerebrum tissue. H&E, 400x.

Table 1: The effect of different dose of fluoxetine on weight of pregnant mice

	Weight (g) (Mean ± SE)	
	7 days	18 days
Control	32.14 ± 1.04	33.12 ± 1.27
60 mg/kg	30.98 ± 1.19	29.96 ± 1.01
80 mg/kg	30.56 ± 0.98	30.01 ± 1.11

### Discussion

The histopathological findings of the present study in the pregnant mice brain showed satisfactory tissue lesions

when treated with fluoxetine at the doses 60 and 80 mg /kg from the 7<sup>th</sup> up to 18<sup>th</sup> days of pregnancy showed clear tissue degeneration and necrosis of the brain tissue, that were consisted with what were indicated by (21) when they treated mice with fluoxetine added to their dietary at the dose of 10 mg/kg for 24 months the drug caused histopathological changes in the central nervous system of the mice, while the results were not agreed with what were indicated by (22). The results also agree with what (23) referred to when they treated adult mice with fluoxetine for 12 weeks the drug cause decrease in the neurogenesis of the subventricular of the brain. The results also consisted with what (24) indicated when they treated rats with fluoxetine with the dose of 10 mg/kg for 1 month added in the drinking water, the treatment showed that there was abnormality presence like necrotic dead neuronal and non-neuronal cells in the brain cortex. The results agreed with what (25) indicated that fluoxetine stimulate changes in the mice brain. The lesions that occur in the present study maybe due to that fluoxetine decrease antioxidant levels and increase oxidant levels by elevating superoxide anion levels and induced oxidative stress, all these consequences may be cause of neuronal damage of the adult brain (13,26) and long-term exposure to the fluoxetine may cause brain tissue damage (27)

As well as the results of the microscopic examination of the mice fetal brain which administered orally with fluoxetine at the doses of 60 and 80 mg/kg from the 7<sup>th</sup> up the 18<sup>th</sup> days of pregnancy showed many lesions represented with degeneration and necrosis of the neurons which were agreed with what were indicated by (28), thus suggesting that the use of antidepressant by pregnant woman can induce alternation in their neural development (19). The results also agreed with what (19) indicated when they injected the mice with 10 mg/kg of fluoxetine intraperitoneally in the days 9<sup>th</sup>, 10<sup>th</sup>, 11<sup>th</sup> of pregnancy the drug caused nervous tissue degeneration, decreased in the neuroglial tissue and wide spaces between neurons. The present results were similar to what (29) referred to when they gave fluoxetine orally to the pregnant rats in the days 1<sup>st</sup>, 4<sup>th</sup>, 8<sup>th</sup>, 12<sup>th</sup>, 18<sup>th</sup> of pregnancy that the brain contained moderate the levels of radioactivity so that the drug can cause damage of the nerve cells in the fetal brain. The lesions that occur in the present study may be due to the, fluoxetine was capable of differentially modulating oxidative stress in the brain (14), so monoaldehydes also increased, which is an index of lipid peroxidation, so that peroxidation activity was also increased this may lead to significant depletion of reduced glutathione so all this consequences can cause changes in the antioxidant levels of the brain which can cause brain tissue damage (30).

The results of the present study showed there was decrease in body weight in both treated groups with the doses 60 and 80 mg/kg compared to the control group. The

results of the present study consistent with what is indicated by (16) when they treated the pregnant mice with the fluoxetine at the doses of 0.05, 0.104, 0.204 mg/kg of B.W. from the 6<sup>th</sup> up to both 15<sup>th</sup> and 19<sup>th</sup> days of gestation as the drug caused reduction in pregnant mice weight. The results also were accordance with the findings of many investigators such as (31-33). While the results of the present study were not consistent with what was indicated by (34) when they injected the mice with the dose of 0.06 mg/kg for 30 days, the drug caused increase in body weight. The reduction of body weight in the present study may be due to that the fluoxetine is affecting intrauterine fetal development so the number of the uteri implantation and embryos were smaller compared to the normal state (35), or may be due to the, that serotonin and neuropeptide Y neurons in the hypothalamus respectively inhibit and stimulate food intake, fluoxetine not only inhibits various aspects of the activity of the neuropeptide Y but also, reduce neuropeptide Y release in the periventricular nucleus a major site of neuropeptide Y release which is highly sensitive to appetite stimulating actions of neuropeptide Y (36).

### Conclusion

Using antidepressants in pregnancy specially during organogenesis has a negative effect on the brain structure of both mothers and embryos, so this may increase the risk of mental illness occurrences specially in the fetus later. Thus, caution should be taken when abusing this drug in critical periods of pregnancy and it should be taken under medical advice to reduce the occurrence of unwanted or dangerous side effects.

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