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THE IMPACT OF THE SYNTHETIC FLUOXETINE AND HERBAL ST.JHON'S WORT ON THE FETUS BRAIN OF DEPRESSED MOTHERS TREATED WITH RESERPINE

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ABSTRACT

Depression has been known as a major public health problem. Women are at greatest risk of suffering from depression during the childbearing years and those may either become pregnant while taking an antidepressant or may need a prescription for one during pregnancy. The present study is aimed to evaluate, the safety of maternal treatment with the synthetic Fluoxetine (Flux) and the herbal St. John's Wort (SJW) on the cerebral cortex of mice fetuses of depressed mothers. Pregnant female mice were injected once subcutaneously (S.C.) by 0.1mg/kg of reserpine on day 7 of gestation (GD 7), and received daily 7.5 mg/kg of Flux or SJW by oral gavage from GD 8 to GD 14.The brain of fetuses were excised and prepared for determination of the content of the monoamine neurotransmitters (5-HT, DA & NE), Malondialdehyde (MDA) content, Nitric oxide (NO) content, Glutathione reduced (GSH) and oxidized (GSSG) contents. Examination of the cerebral cortex of 18-days-old fetuses maternally received Res + Flux revealed some variations from control. While, the cerebral cortex of 18-days-old fetuses maternally received Res + SJW showed a disruptive appearance of the neuroepithelium compared with that of the normal control fetuses. Both Flux and SJW cross the placenta of mothers and cross the brain barrier of fetuses and revealed remarkable alternations on fetuses. The results showed that treatment with Flux or SJW improve the monoamine contents especially in the brain stem and the oxidative markers.

KEYWORDS: Fluoxetine, St.Jhon's Wort, depression, the cerebral cortex, monoamine neurotransmitters, MDA, NO, GSH, GSSG.

INTRODUCTION

Depression is a very common and a highly heterogeneous psychiatric disorder with symptoms including deficits of cognitive, psychomotor, and profound sadness, or loss of interest in activities (Anhedonia). It can be a serious life threatening condition as depression may lead the affected people to a persistent sad, feeling of hopelessness, changes in sleep and appetite, difficulty of concentration and making decisions, and recurring thoughts of death or suicide. Thus, depression significantly interferes with the normal functional ability of affected people **(Fanselow and Ledoux, 1999 and Fava and Kendler, 2000).**

Depression has been known as a major public health problem. It is well known that monoamine neurotransmitters, such as dopamine (DA), noradrenaline (NA), and serotonin (5- HT) in the central nervous system play a key role in the pathophysiology of depression **(Mayeux *et al.*, 1984; Colpaert, 1987; Cantello *et al.*, 1989; Chan-Palay and Asan, 1989; Elhwuegi, 2004).**

Reserpine treatment resulted in depression syndrome and Parkinson's disease as side- effects in 15% of the treated patients, and in animal models depression like syndrome showing motor retardation. Reserpine causes depression because it is a specific irreversible inhibitor of vesicular amine pump so it prevents the uptake of catecholamines which leads to depletion of catecholamine stores **(Kandle *et al.*, 1991).**

Depression is more common in women than men across all age groups and cultural backgrounds **(Kessler *et al.*, 1993).** Twenty percentage (20%) of pregnant women report symptoms of depression during pregnancy **(Patkar *et al.*, 2004).** Treatment with antidepressants cannot be avoided in some pregnant women. The decision of prescribing and taking psychotropic drugs during gestation and lactation is a difficult task due to the rarity of studies investigating the safety of these drugs for the babies, especially for their long-term neurobehavioral development **(Zeskind and Stephens, 2004).** Antidepressant use during pregnancy has increased 300% from 1998 to 2005 according to The National Birth Defects Prevention Study in the United States **(Alwan *et al.*, 2011).**

Selective serotonin reuptake inhibitors (SSRIs) are the most widely prescribed antidepressants worldwide because of their efficacy, relatively few side-effects, and therapeutic safety **(Barbey and Roose, 1998).** SSRIs such as Fluoxetine (commercially known as Prozac, Prozac Weekly, Sarafem, Rapiflux, or Selfemra) is widely used for the treatment of depressive disorders in women during pregnancy and the postpartum period **(Wong and Bymaster, 1995).**

Fluoxetine acts as antidepressant drug that increases the plasma serotonin levels and crosses the placenta, so it affects the fetus by reduction in uterine blood flow, which leads to decrease of oxygen and nutrients delivery to the fetus, reduce growth, elicite preterm delivery, increase high-voltage/non-rapid eye movement behavioral state in the fetus and may interfere with normal fetal neurodevelopment **(Kallen, 2004 and Morrison *et al.*, 2005).** Fluoxetine exposure at late gestation resulted in a greater incidence of preterm delivery, approval to special care nursery, poor neonatal adaptation and decrease birth weight. This influence may be related to the time of gestation at which the fetus was exposed and the duration of exposure. So fluoxetine exposure at first and third trimesters affects fetal outcome **(Cohen *et al.*, 2000).**

St John's Wort (*Hypericum perforatum*), named because this herb flowers around John the Baptist's day in June and "Wort" is derived from the German word for plant, is the most common herbal therapy used for depression. In Germany, **Müller and Kasper (1997)** reported that it was accounted for a quarter of all anti- depressant prescriptions. There is no data on reproductive safety of St John's Wort, despite the widespread availability and extensive research on the herb **(Goldman *et al.*, 2003).** *Hypericum perforatum* is vastly used in folk medicine for the treatment of depression. Recently, studies with animal models for antidepressive drug screening have revealed this putative effect **(Butterweck *et al.*, 1997).***Hypericum* extracts containing high concentration of flavonoid can modulate the tone of several neurotransmitters in the brain and inhibit the monoamine oxidases **(Bladt and Wagner, 1994).**

The selective serotonin reuptake inhibitors (SSRIs) act primarily by blocking neuronal 5-HT reuptake, because Serotonin (5-HT) transmission appears to be involved in the physiopathology of depressive disorders as well as in the therapeutic action of antidepressant drugs. Acute administration of a SSRI produced a small and transient increase in extracellular 5-HT concentrations in the rat frontal cortex **(Blier and De Montigny, 1994).** Monoamine theory of depression are believed to be the functional deficiency of catecholamine and 5-hydroxytriptamine (5-HT) in the brain **(Elhwuegi, 2004).**

The aim of this study is to evaluate, the safety of maternal treatment with the synthetic Fluoxetine and the herbal St. John's Wort on cerebral cortex of mice fetuses of depressed mothers. The brain of fetuses were excised and prepared for determination of the content of the Monoamine neurotransmitters (5-HT, DA & NE) contents, Malondialdehyde (MDA) content, Nitric oxide (NO) content, Glutathione reduced (GSH) and oxidized (GSSG) contents.

MATERIAL AND METHODS

Animals

Eighty female mice were used with an average body weight of 25-30g, they were obtained from Theodor Bilharz Research Institute (TBRI). Upon arrival the animals were housed in plastic cages and kept under conditions of regular 12/12 hours light- dark cycles. They were fed on commercial platted diet. Vitamins were added as fresh vegetables and the animals were provided with milk and water *ad libitum*. All animal experimental protocols were approved by Committee of Scientific Ethics of Ain Shams University and were carried out in accordance with its guidelines for animal use.

Drugs

Reserpine, it was purchased from sigma Aldrich, USA.

Fluoxetine, it is known by the brand name PROZAC. Manufactured by: Patheon France 40, boulevard de Champaret 38300 Bourgoin-Jallieu, France for Eli Lilly France S.A.S.

St. Jhon's Wort, it is known by the brand name Safamood. It was purchased from pharma Atos Company, Cairo, Egypt.

Doses and Injections

Reserpine was dissolved as 0.1 mg/ml (wt/v) in (0.5 % v/v) glacial acetic acid and was given to the mice as a single dose of 0.1 mg/kg of the body weight according to **Abou-Nour *et al.* (2017)** subcutaneously (S.C.) at the day 7 of gestation.

Fluoxetine, was dissolved in tap water and given daily to the mice at a dose of 7.5 mg/kg of body weight according to **Bairy *et al.* (2006)** by oral gavage from GD 8 to GD 14.

St. John's Wort, was dissolved in tap water and given daily to the mice at a dose of 70 mg/kg of body weight according to **Bach-Rojecky *et al.* (2004)** by oral gavage from GD 8 to GD 14.

Experimental Design

Eighty female mice were divided into four main experimental groups. Each group consisted of about (8-10) pregnant females mice. Normal control, control depressed (Res group), Fluoxetine (Res + Flux) group and St. John's Wort (Res + SJW) group. The pregnant females of all groups were sacrificed at the day 18 of gestation by decapitation. Females of both control and treated groups were dissected. The uteri were removed, dissected in normal saline solution and the fetuses were obtained for morphological and histological examinations. Living fetuses were distinguished from dead ones by their spontaneous movement. The brain of fetuses were excised and prepared for the determination of cortex and brain stem Monoamine neurotransmitters content, and the whole brain Malondialdehyde (MDA) content, Nitric oxide (NO) content, Glutathione reduced (GSH) content and Glutathione oxidized (GSSG) content.

Histological Examination

The fetuses of both control and treated groups were dissected, the cerebral cortex was fixed in aqueous Bouin's solution for 48 hours, transferred to 70% alcohol, dehydrated in ascending ethyl alcohol series, cleared in Terpineol and embedded in paraffin wax. The paraffin blocks were sectioned, stained with haematoxylin and eosin and several photomicrographs were taken as required.

Biochemical Assays

The brain was immediately excised on jacket ice. The frontal cortex and brain stem, were separated according to **Swanson (2004)** and then rapidly transferred into a refrigerator (-80)°C pending HPLC analysis. The whole brain tissues were cut longitudinally and the first half of the tissues were homogenized in iced 10% potassium chloride for homogenization then followed by centrifugation in cooling centrifuge at 4 °C for 20 min at 5000 rpm and the supernatant was obtained, for determination of Malondialdehyde (MDA) content according to **Karaatepe (2004)**, Glutathione (GSH and GSSG) contents according to **Jayatilleke and Shaw (1993)** and Nitric oxide (NO) content according to **Papadoyannis *et al.* (1999)** by High Performance Liquid Chromatography (HPLC). The second half of the brain tissues were separated into the cortex and the brain stem were immediately homogenized in 70% HPLC grade methanol using a homogenizer surrounded with an ice jacket. The homogenates were used for the determination of the cortex and brain stem contents of the monoamine neurotransmitters (Norepinephrine, Dopamine, and Serotonin) contents according to **Pagel *et al.* (2000)** by HPLC.

Statistical Analysis

Numerical data were expressed as means ± standard errors of mean. The significance of the interrelation of the treated groups to the control was tested using one way analysis of Variance, ANOVA and Tukey's multiple comparison test. Using Statistical Package for Social Sciences (SPSS V.23).

RESULTS AND DISCUSSION

The histological sections of the cerebral cortex of 18- days- old control fetuses can be differentiated into six cortical layers; from the superficial to the deep surface are: molecular layer, outer granular layer, outer pyramidal cell layer, inner granular layer, inner pyramidal cell layer and the polymorphic layer (Fig. 1). The first layer, molecular layer, consists mostly of nerve fibers beside some neuroglial cells containing spherical nuclei. The second layer, outer granular layer, shows numerous pyramidal neurons with darkly stained nuclei. Numerous glioblasts are usually scattered inside this area. Such cells are characterized by their small spherical nuclei. The third layer, outer pyramidal cell layer, consists of pyramidal cell bodies of neuroblasts. This layer is not sharply distinguished from the second layer. However, the nerve cells of this layer are somewhat larger and possess a typical pyramidal shape. The fourth layer, inner granular layer, is characterized by the presence of many small stellate neurons as well as numerous neuroglia cells (Fig. 2).The fifth layer, inner pyramidal cell layer, contains pyramidal cell bodies with dark rounded or oval shaped nuclei. Finally, the polymorphic layer, contains cells with many shapes; spindle shaped or fusiform neuroblasts possessing oval shaped nuclei (Fig. 3).

The cerebral cortex of 18-days-old fetuses of Res group revealed no remarkable changes as compared with that of the control (Fig. 4-6). On the other hand, the cerebral cortex of 18-days-old fetuses of Res + Flux group revealed some variations from control (Fig. 7). The neuroglia cells in the molecular layer exhibited densely stained pyknotic nuclei (Fig. 8). The neuropile of the outer granular layer and outer pyramidal layer contained wide vacuoles and many of the neuroblasts possessed small pyknotic nuclei. The pyramidal cells in the outer pyramidal cell layer showed sign of pyknosis and karyolysis of their nuclei (Fig. 8).The pyramidal cells of inner granular layer are necrotic and shrunken (Fig. 9). The pyramidal cells of inner pyramidal cell layer showed vacuolation in the cytoplasm of many stellate and pyramidal neurons and also showed pyknotized and karyolysed nuclei (Fig.9). The fusiform and pyramidal cells of the polymorphic layer showed pyknotized nuclei (Fig. 9).

The cerebral cortex of 18-days-old fetuses of Res + SJW group showed a disruptive appearance of the neuroepithelium compared with that of the normal control fetuses (Fig. 10). The different layers of the neuroepithelium contained wide vacuoles and necrotic neuroglia cells and shrunken (Fig. 11).The neuroblasts in the outer granular layer showed signs of pyknosis and karyolysis of their nuclei (Fig. 11). The pyramidal cells of the outer pyramidal cell layer, the inner granular layer and inner pyramidal cell layers showed sign of pyknosis and karyolysis of their nuclei (Fig. 12). The polymorphic layer possessed numerous vacuoles and the nuclei of most pyramidal cells were pyknotized and karyolysed (Fig. 13).

The neuropil is defined as the space between neuronal and glial cell bodies comprising dendrites, axons, synapses, glial cell processes, and microvasculature **(Spocter *et al.*, 2012).**

In support to the present study, **Maes *et al.* (2011)** stated that Reactive Oxygen Species (ROS) may react with macromolecules of the cell like fatty acid, DNA, protein, etc thereby causing harm to these macromolecules, when these radicals become in excess or when the antioxidant system gets consumed. Brain, due to its high metabolic rate, is one of the most vulnerable organs to the damaging effects of ROS. This may explain ROS involvement in several neuropsychiatric diseases. Reactive Oxygen Species (ROS) is increased in plasma in patients with major depression, may lead to membrane degradation, cellular dysfunction and apoptosis **(Bilici *et al.*, 2001).** The risk of neurodevelopmental disorders and behavioral deficits increased in developing fetuses and newborns after fluoxetine treatment **(Oberlander *et al.*, 2009).** Also, **Oliveira *et al.* (2016)** reported that oxidative stress plays an important role in the development of neurodegenerative diseases, such as Alzheimer's and Parkinson's disease and stroke. Apoptosis and excitotoxicity are among the mechanisms that cause neuronal death, involving ROS and reactive nitrogen species (RNS). **Hendrick *et al.* (2003)** stated that fluoxetine crosses the placental barrier of humans and rats.

The cortex and brain stem Norepinephrine (NE), Dopamine (DA) and Serotonin (5- HT) of the fetuses of different experimental groups were illustrated in Table (1).There was a significant decrease in the cortex and brain stem NE contents in the fetuses of all treated groups except the brain stem and cortex NE of Res + SJW fetuses showed a non-significant decrease as compared to the fetuses of the control group. Whereas a significant increase in the brain stem NE ($p \leq 0.05$) in the fetuses groups treated with Res + SJW or Res + Flux when compared with the fetuses of the Res treated group. Moreover, brain stem NE contents of the fetuses of Res + SJW treated mice were significantly increased in comparison with the fetuses of Res +

Flux group.

Concerning cortex and brain stem DA contents, there was a significant decrease in the fetuses of all treated groups except the brain stem and cortex DA contents of Res + SJW fetuses exhibited a non-significant decrease as compared to the fetuses of the control group. Whereas a significant increase was reported in brain stem and cortex DA ($p \leq 0.05$) in the fetuses groups treated with Res + SJW or Res + Flux when compared with the Res fetuses.

There was a significant decrease in brain stem 5HT contents in the fetuses of the Res group compared to the fetuses of the control group. On the other hand, data revealed non-significant change in brain stem and cortex 5HT in all fetuses groups when compared to the fetuses of the control group. Moreover, there was a significant increase in brain stem 5HT contents in Res + SJW embryos as compared to the Res treated embryos.

Depression can be raised when low serotonin levels promote low levels of norepinephrine, another monoamine neurotransmitter **(Shah *et al.*, 1999).** Some antidepressants also enhance the levels of norepinephrine directly, whereas others raise the levels of dopamine. These observation gave rise to the monoamine hypothesis of depression **(Nutt, 2008).**

In support to the present study, **Meyer *et al.* (2006)** found that most of serotonergic , noradrenergic and dopaminergic neurons are located in mid brain and brain stem nuclei and project to large areas of the entire brain. **Becker *et al.* (1997)** stated that acute administration of *Hypericum* extracts increase noradrenaline levels in the diencephalon. In the same trend, extract ph-50, containing more flavonoids, also increased the noradrenaline content of the brainstem. In the light of recent findings suggesting a role of the brainstem in the control of mood. Fluoxetine was found to elevate the 5HT levels in the striatum with no changes in the levels of DA **(Perry and Fuller, 1992).**

The current results are parallel with **Capello *et al.* (2011)** who found that fetal CNS exposure is similar to that of the pregnant dam, although we observed variable placental transfer. Antidepressants are able to cross the placenta and relevant concentrations have been revealed in umbilical vein blood **(Hendrick *et al.*, 2003). Baumann and Rochat (1995)** also stated that SSRIs are able to pass the blood brain barrier.

In addition, **Boer *et al.* (2010)** stated that the best known mechanism of antidepressant drug action is the inhibition of neuronal reuptake, by which the extra neuronal monoamine

concentrations are raised and thus noradrenergic and serotonergic neurotransmission are induced. The antidepressant fluoxetine is a selective serotonin reuptake inhibitor (SSRI), which raises serotonin neurotransmission **(Morrison *et al*., 2005).** Also, **Kumar *et al*. (2009)** found that many brain regions, especially those in the limbic system such as hippocampus and frontal cortex, due to their sensitivity to metabolic insults, act in concert to mediate the symptoms of depression accompanied with pain.

In supporting to present result, **Favaro *et al*. (2008)** found that 5- HT levels raised during brain development due to maternal Flux exposure could induce pups' monoaminergic neurotransmission development.

Oxidative stress is an imbalance between generation and elimination of reactive oxygen species (ROS) and reactive nitrogen species (RNS). The Central Nervous System (CNS) is known for being particularly sensitive to both and this sensitivity is due to susceptibility of the brain is caused by a high metabolic rate, a low concentration of glutathione and antioxidant enzyme catalase (CAT) and a high proportion of polyunsaturated fatty acids **(Oliveira *et al*., 2016).**Glutathione plays an important protective role against oxidative stress in the brain, because it is the most abundant thiol compound in cells of all organs **(Gawryluk *et al*., 2011).** Depressed people have lower levels of antioxidant enzymes and consequently high levels of reactive oxygen species (ROS) **(Moylan *et al*., 2014).**

Results of brain MDA of the fetuses of different experimental groups were illustrated in Table (2). MDA significantly increased ($p \leq 0.05$) in the Res group as compared to the control group. On the other hand, data revealed a non-significant increase in the brain MDA in other groups as compared to the control group.

Results of NO of the fetuses of different experimental groups were illustrated in Table (2). The data revealed a non-significant increase in NO in the all treated groups as compared to the control group.

Results of cortex GSH, GSSG and GSH/GSSG ratio were recorded in Table (2). A significant decrease was shown in GSH and GSH/GSSG in the fetuses of the Res group, GSH/GSSG ratio in the fetuses of Res + SJW group and GSH in the fetuses of Res + Flux group as compared to the fetuses of the control group. On the other hand, data revealed a non-significant change in GSH in the fetuses of Res + SJW group, GSH/GSSG ratio in the fetuses

of Res + Flux group and GSSG in the fetuses of all treated groups as compared to the fetuses of the control group.

Moreover, the fetuses of Res + Flux group and the fetuses of Res + SJW group exhibited a significant increase in GSH contents as compared to the fetuses of the Res treated group. While GSSG contents in the fetuses of Res + SJW group was significantly increased as compared to the fetuses of Res + Flux treated group.

In supporting to the present result, **Galecki *et al.* (2009)** stated that patients with depression are characterized by an increase in MDA levels and other products of lipid peroxidation. Oxidative stress in restraint animals was reduced after administration of fluoxetine **(Zafir and Banu, 2007).** Also, **Kotan *et al.* (2011)** demonstrated that MDA concentrations in depressed patients are by-and-large raised compared to healthy control groups. MDA also was decreased significantly following 24 weeks of antidepressant treatment. **Moskovitz *et al.* (2002)** suggested that ROS are implicated as major causes of cellular injuries in a variety of clinical abnormalities including neurodegenerative diseases such as ischemia, Alzheimer's disease (AD), Parkinson's disease (PD). **Bajpai *et al.* (2014)** found that there was a significant increase in malondialdehyde (MDA) level in the patients with major depression as compared to healthy control. Increased MDA levels implicate in increased lipid peroxidation products in major depressive disorder. MDA status is used as biomarker for oxidative stress. It has been reported that elevated MDA was connected with different depressive disorder, like auditory-verbal working memory, impairment of visual-spatial, short-term and delayed declarative memory **(Maes *et al.*, 2011; Kotan *et al.*, 2011; Talarowska *et al.* 2012).**

The current results are in coincidence with, **Gawryluk *et al.* (2011)** who suggested that GSH levels are lower in postmortem prefrontal cortex from patients with bipolar disorder (BD), major depressive disorder (MDD), and schizophrenia (SCZ) which can compromise the antioxidant capacity. There was a raise in the Reactive Oxygen Species (ROS) in the stressed group and treatment with fluoxetine inverted the effect through the amelioration in the level of antioxidants like SOD, CAT and GSH **(Novío *et al.*, 2011).**

Moreover, **Jayakumar *et al.* (2017)** found that the levels of antioxidants are improved significantly after fluoxetine treatment. *Hypericum perforatum* administration significantly reduced lipid peroxidation, nitrite concentration and partially restored GSH and catalase activity in chronic restrained mice suggesting a strong antioxidant effect **(Kumar *et al.*,**

2010).

The level of GSH raised by fluoxetine and by the higher dose of sertraline **(Abdel Salam *et al.*, 2013).**

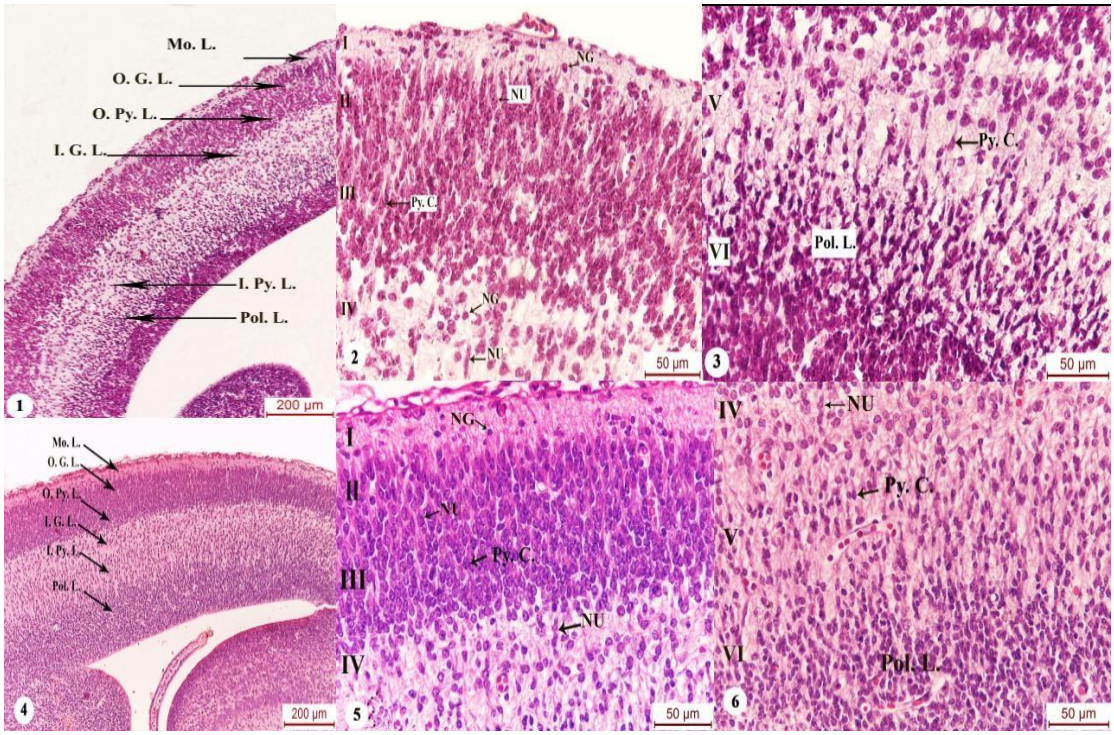

Fig. 1: Photomicrograph of a section of the prosencephalon of 18-days- old control fetus showing the different layers of the cerebral cortex. Molecular Layer (Mo. L.), Outer Granular Layer (O. G. L.), Outer Pyramidal cell Layer (O. Py. L), Inner Granular Layer (I. G. L.), Inner Pyramidal cell Layer (I. Py. L.) and Polymorphic cell Layer (Pol. L.). Fig. 2: Enlarged portion of Fig.1 showing I, II, III & IV layers containing neuroglia cells (NG), neuroblasts (NU) and pyramidal cell bodies (Py. C.). Fig. 3: Enlarged portion of Fig.1 showing V&VI layers containing pyramidal cell bodies (Py. C.) and showing the polymorphic cell layer (Pol. L.) of the cerebral cortex. Fig. 4: Photomicrograph of a section of the cerebral cortex of Res group showing different layers of cerebral cortex. Molecular Layer (Mo. L.), Outer Granular Layer (O. G. L.), Outer Pyramidal cell Layer (O. Py. L), Inner Granular Layer (I. G. L.), Inner Pyramidal cell Layer (I. Py. L.) and Polymorphic cell Layer (Pol. L.). Fig. 5: Enlarged portion of Fig.4 showing I, II, III & IV layers containing neuroglia cells (NG), neuroblasts (NU) and pyramidal cell bodies (Py. C.). Fig. 6: Enlarged portion of Fig.4 showing V&VI layers containing pyramidal cell bodies (Py. C.), neuroblasts (NU) and showing the polymorphic cell layer (Pol. L.) of the cerebral cortex.

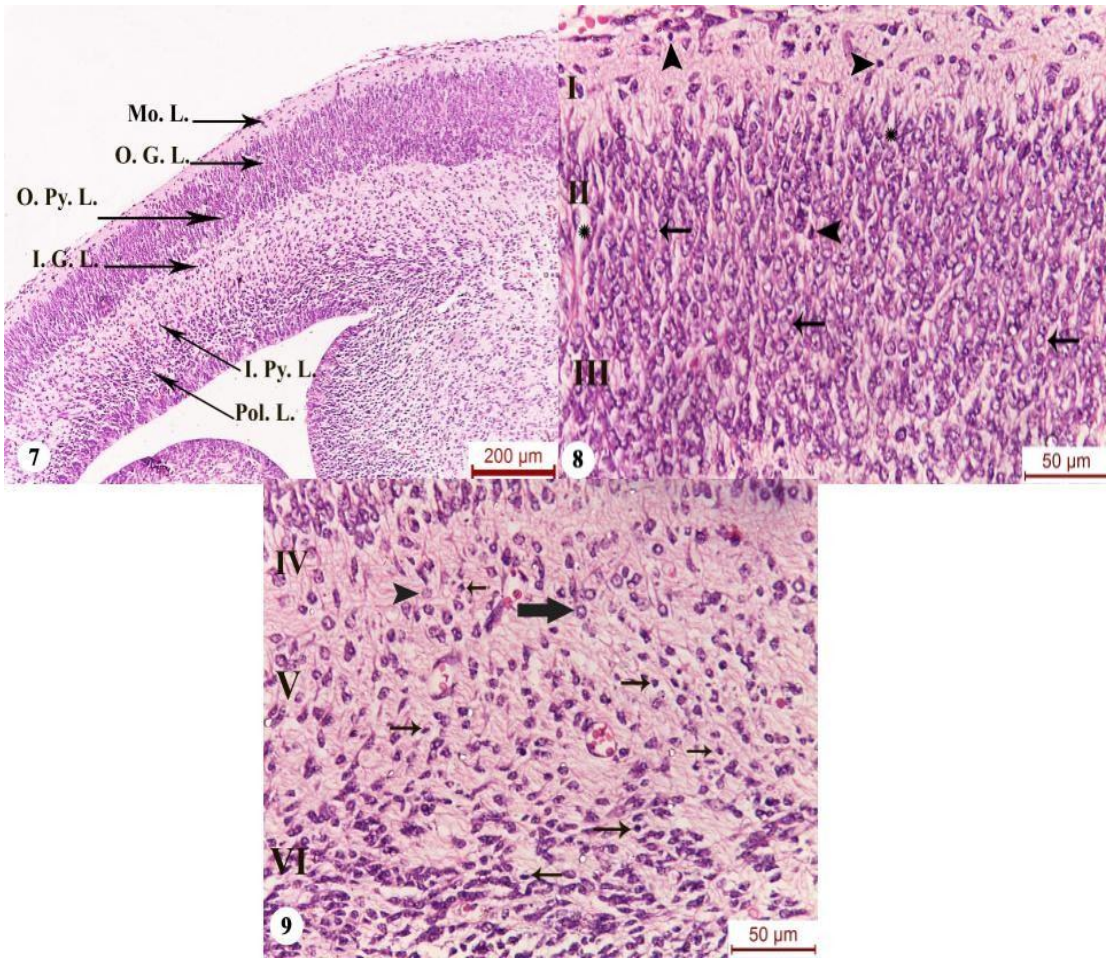

Fig. 7: Photomicrograph of a section of the cerebral cortex of (Res + Flux) group showing the disruptive appearance of the neuroepithelium. Molecular Layer (Mo. L.), Outer Granular Layer (O. G. L.), Outer Pyramidal cell Layer (O. Py. L), Inner Granular Layer (I. G. L.), Inner Pyramidal cell Layer (I. Py. L.) and Polymorphic cell Layer (Pol. L.). Fig. 8: Enlarged portion of Fig.7 showing pyknotized nuclei of the neuroglia cells of the molecular layer (arrowheads). Pyknotized (arrowheads) and karyolysed nuclei (arrows) in the neuroblast cells of the II layer and the vacuolation of neuropile (stars). Fig. 9: Enlarged portion of Fig.7 the pyramidal cells of IV and V layers showing pyknotic (arrows) karyolysed (thick arrow) nuclei and necrotic cells (arrowheads). The VI layer (polymorphic cell layer) showing pyknotized nuclei (arrows).

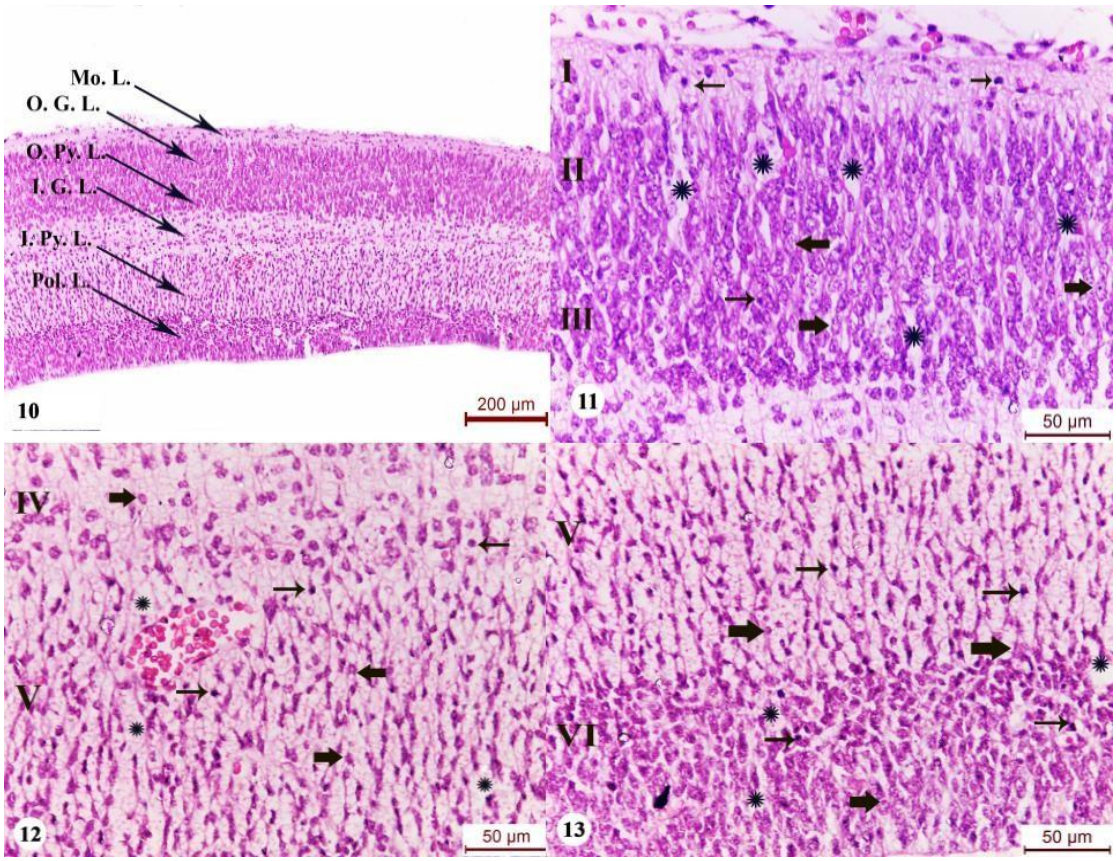

Fig. 10: Photomicrograph of a section of the cerebral cortex of (Res + SJW) group showing the disruptive appearance of the neuroepithelium. Molecular Layer (Mo. L.), Outer Granular Layer (O. G. L.), Outer Pyramidal cell Layer (O. Py. L), Inner Granular Layer (I. G. L.), Inner Pyramidal cell Layer (I. Py. L.) and Polymorphic cell Layer (Pol. L.). Fig. 11: Enlarged portion of Fig. 10 showing vacuolation of the neuropile of I&II layers (stars). Pyknotized nuclei (arrows) of neuroglia cells. Pyknotized (arrows) and karyolysed (thick arrow) of the neuroblast cells of the II layer. Fig. 12: Enlarged portion of Fig. 10 showing vacuolation of the neuropile of IV&V layers (stars). Pyknotized (arrows) and karyolysed (thick arrows) nuclei of the pyramidal cells of IV&V layers. Fig. 13: Enlarged portion of Fig. 10 showing pyknotized (arrows) and karyolysed (thick arrows) nuclei of the pyramidal cells of V layer. Vacuolation of polymorphic cell layer (stars) with highly pyknotized (arrows) and karyolysed (thick arrows) nuclei.

Table 1: Showing cortex and brain stem neurotransmitters Norepinephrine (NE), Dopamine (DA) and Serotonin (5HT), (µg/g tissue) of the fetuses of different treated groups.

Brain area	Parameter groups	NE	DA	5HT
Cortex	Control	0.37±0.020	1.25±0.116	0.19±0.014
	Res	0.28±0.012[a]	0.66±0.044[a]	0.17±0.006
	Res + Flux	0.32±0.015[a]	0.98±0.112[ab]	0.18±0.019
	Res + SJW	0.33±0.018	1.04±0.057[b]	0.16±0.014
Brain stem	Control	0.28±0.011	1.11±0.070	0.11±0.010
	Res	0.10±0.013[a]	0.54±0.044[a]	0.04±0.016[a]
	Res + Flux	0.15±0.014[ab]	0.80±0.066[ab]	0.08±0.025
	Res + SJW	0.26±0.017[bc]	0.95±0.045[b]	0.09±0.004[b]

Values are means±SE of 6 fetuses mice at p≤0.05, a=significant change from control, b= significant change from Reserpine (Res), c=significant change from Fluoxetine (Flux).

Table 2: Showing cortex Malondialdehyde (MDA) (nMol/g), Nitric oxide content (µMol/g) and brain Glutathione contents (mg/g) of the fetuses of different treated groups.

Groups	MDA	NO	GSH	GSSG	GSH/GSSG
Control	1.74±0.091	1.13±0.023	2.44±0.054	0.13±0.015	20.01±2.942
Res	2.49±0.188[a]	1.27±0.080	1.69±0.056[a]	0.14±0.008	12.79±1.019[a]
Res + Flux	2.09±0.109	1.23±0.145	2.13±0.021[ab]	0.12±0.011	18.21±1.849
Res + SJW	2.09±0.292	1.22±0.139	2.22±0.157[b]	0.16±0.005[c]	13.87±0.555[a]

Values are means±SE of 6 fetuses mice at p≤0.05, a=significant change from control, b= significant change from Reserpine (Res), c=significant change from Fluoxetine (Flux).

CONCLUSION

Either Flux or SJW improves the depressed effect of reserpine on the fetuses of treated mice but they still had a negative impact on the histology and biochemistry on the fetuses brain development.

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