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Comparative study between *Hyoscyamus muticus* plant and Anafranil on frontal cortex of pregnant albino rats and their offspring

Ahmed S. Alazzouni*[1], Asmaa A. Elenany[1], Mohamed S. Gabri[1], Dina H. Abd el-kader[2], Ashraf Al-Barakati [3] and Basma N. Hassan[1]

[1]Department of Zoology & Entomology, Faculty of Science, Helwan University, Cairo, **Egypt**
[2]Department of Histology, Faculty of Medicine, Cairo University, Cairo, **Egypt**
[3]Department of Human Anatomy, Faculty of Medicine, Taif University, **Saudi Arabia**

***Correspondence:** drahmedalazzouni@gmail.com **Received 06-08-2020, Revised: 28-11-2020, Accepted: 02-12-2020 e-Published: 20-12-2020**

The possible antidepressant role of *Hyoscyamus muticus* on the damage of the frontal cortex for gestational depression on mother and offspring. The present work was to evaluate the toxicological effect of *Hyoscyamus muticus* on frontal cortex of the brain tissue of pregnant female rats, and comparing these changes with those induced by anti-depressant drugs. 25 adult female and ten male albino rats were mating and divided into five groups: Control non-pregnant, pregnant, postpartum, *Hyoscyamus* treated & Anafranil treated groups. *Hyoscyamus* extract and drug (Anafranil) were administered in dose of dose of 17.5-25 mg and 15mg/kg respectively orally for rat from the first day of fertilization to parturition. respectively for rat orally from the first day of fertilization to parturition. The frontal cortex were processed and stained by H&E, Congo red & immunoreaction of SERT and Transmission Electron Microscopy. Morphometric analysis for thickness of cortex and immunoreaction area percent were performed and statically analyzed. Results: *Hyoscyamus* showed more improvements than Anafranil for mothers and offspring. Anafranil showed normal cells, congested blood vessels and mild amount of amyloid plaques. TEM showed degenerated mitochondria and extensive fragmented RER in mothers also, decreased size of layers, normal granular and pyramidal cells. TEM showed nucleus with fragmented chromatin, degenerated mitochondria, fragmented rough endoplasmic reticulum RER and vacuolated cytoplasm in off springs. Conclusion: *Hyoscyamus* treatment exerted ameliorated effects against pregnancy-induced damage on mother while not offspring, but Anafranil showed some deleterious effects on mother and offspring.

Keywords: Pregnancy– Depression– Frontal cortex– *Hyoscyamus muticus* –Antidepressant–Anafranil

INTRODUCTION

Pregnancy is regarded as a period of sex hormone excess but is more complex than the other reproductive stages from psychiatric and endocrine perspectives. First, not only do estrogen and progesterone increase during pregnancy, but other hormones such as cortisol and corticotrophin-releasing hormone (CRH) increase, and the immune response is altered (Skalkidou et al. 2012). Second, pregnancy is a period with increased risk for relapse of depression (Cohen et al. 2006). Gestational depression, depression is a worldwide public health problem and is considered one of the leading causes of disability. In women, depression is even more disturbing when it occurs during gestation; according to certain authors, the

neuroendocrine changes that occur during gestation favor the development of depressive disorders (Vieira et al. 2013). Narcotic plants that induce hallucinations are variously called hallucinogens (hallucination generators), psychotropics, and psychedelics (*Prance and Nesbitt,* 2005). These hallucinogens are a group of chemically heterogeneous compounds, all with the ability to induce altered states of consciousness characterized by alterations in mood (Vollenweider et al. 2000). Anticholinergic hallucinogens such as scopolamine (the main constituent in the Datura genus, Hyoscyamus) and several other genera of plants (Galanter et al. 2015).

Hyoscyamus muticus, commonly known as the egyptian henbane, it is a shrub belongs to family solanaceae, is a rich source of tropane alkaloids which have mydriatic, anticholinergic and antispasmodic properties.Hyoscyamine represents 90% of the total alkaloids in addition to small amounts of hyoscine in this plant (Abdelrazik et al. 2019). Antidepressant drugs are indicated for the treatment of depression, anxiety disorders and stress disorder (Keks et al. 2016). Clomipramine is one of the most used TCA drugs, it is a tricyclic serotonin reuptake blocker used in the treatment of depression (Colavito et al. 2011). It contains two benzene rings in its chemical structure (El-Fiky et al. 2016).

Hyoscyamine (daturine) is an ester of tropic acid with atropine, being an amino alcohol. The molecular formula is C17H23NO3. After the plant is dried, the structural composition is modified and hyoscyamine goes through the levogirous isomer called atropine. Hyoscyamine is an antagonist of muscarinic acetylcholine receptors (fig.6) having 98% of the anticholinergic power of atropine (Carpa et al. 2017).

Atropine is a tropane alkaloid, isomer of hyoscyamine. It is found in different concentrations in Datura stramonium and Hyoscyamus. The molecular formula is C17H23NO3 (Behçet, 2014).

The mechanism of action of tropane alkaloids (fig.7) relates to their competitive antagonism at muscarinic acetylcholine receptors, preventing the binding of acetylcholine (*EFSA*, 2008) and thus block the action of Ach at muscarinic neuroeffector sites on smooth and cardiac muscle, gland cells, in peripheral ganglia and in the central nervous system (CNS) (*Gupta*, 2018).

In medicine they are used to relieve the symptoms of Parkinson's disease, to dilate the pupils, increase the heart rate, to cause smooth muscle relaxation, and to reduce secretion such as sweat and gastric acid (*Sevon et al.,* 2001).

Scopolamine has a stronger effect on the central nervous system than hyoscyamine, but causes fewer undesirable side effects and is used for the treatment of motion sickness and the production of derivative drugs for gastric disorders **(***Sevon* et al. 2001**)**.

MATERIALS AND METHODS

Drugs:

An antidepressant drug (Anafranil) in its therapeutic dose. Clomipramine is available in capsule form, as a hydrochloride salt, with dosages of 25 mg, 50 mg, and 75 mg. It produced by Novartis Company, Cairo, Egypt. It is the hydrochloride salt of a phenylpiperidine compound. The dose was calculated according to Aitchison et al. (2010) therefore each animal in Clomipramine group received a daily dose of 15 mg/kg orally. Each dose was dissolved in 0.5 ml distilled water.

Extraction of *Hyoscyamus muticus* leaves:

The leaves of *Hyoscyamus muticus* obtained from Ornamental Plants Department, Faculty of Agriculture, Cairo University; the aqueous extracts of *Hyoscyamus* leaves were prepared using 200g of dried leaves that were ground and the obtained powder was mixed with 1 L of boiling distilled water for 1 hour. The obtained mixture was filtered and preserved in an air-tight dark colored glass container (Alghazeer et al. 2012). The dose was 17.5 mg/kg b.w of the extract dissolved in distilled water was given orally via gastric tube to each rat every day throughout the duration of pregnancy to *Hyoscyamus* treated group (Reza et al.2009).

Experimental animals:

Twenty adult female and ten male albino rats (Rattus norvegicus), weighing 120-150 gm and five female albino rats weighing 70-90 gm. The animals were obtained from the farm of the Egyptian Organization of Biological products and Vaccines in Helwan, Cairo. All animals were housed in standard cages, five per cage, in a controlled temperature room (22ºC), with a 12 h light: 12 h dark cycle. the animals received food and water *ad libitum*.

Experimental design:

The rats were divided into 5 groups: I, II, III, IV, and V (5 mothers and offspring's rats each)

I-Control non- pregnant group :

Consisted of 5 adult female weighing 70-90 gm (non pregnant) rats, they did not receive any drug.

II-Control pregnant group :

Consisted of 5 pregnant rats for 2 weeks and fetus, they did not receive any drug.

III-Postpartum group:

Consisted of 5 mother rats and offspring, they did not receive any drug.

***IV- Hyoscyamus* treated group:**

Consisted of 5 mother rats and offspring, each mother rat was administered with 17.5 mg of *H. muticus* extract orally from the first day of fertilization to parturition.

V-Anafranil treated group:

Consisted of 5 mother rats and offspring, each mother rat was administered with 15 mg/kg of clomipramine orally from the first day of fertilization to parturition.

One week after delivery all treated mother rats and offspring were sacrificed and then dissected for excision of brain that was fixed in Blouin's fluid for 24 hours. They were then subjected to the normal procedures for paraffin blocks formation, sectioned, and stained with hematoxylin & Eosin (*Bancroft and Gamble,* 2002) Congo red and immunoreaction of serotonin transporter for light microscopic examinations (*Rajamohamedsait and Sigurdsson*, 2013).

For Transmission Electron Microscopy, brain tissue samples were collected and processed. Finally, it was examined and photographed using JEOL-JEM-1010 transmission electron microscope at EM unit in The Regional Center For Mycology and Biotechnology, Al-Azhar University *(Afifi and Embaby,* 2016).

Morphometrical studies:

Morphometric measurements were performed using "Leica Qwin 500 C" image analyzer computer system (Cambridge, England) present in Histology Department, Faculty of Medicine, and Cairo University. The area percent for amyloid plaques deposition in frontal sections by congo red stain, fibrosis stained by Masson's trichrome and positive serotonin immunoreaction was measured in 10 non overlapping fields for every specimen at magnification X 400 for all groups.

Statistical analysis:

Results were expressed as the mean ± standard deviation (SD). Using the statistical program statistical package program (SPSS version 25), data for multiple variable comparisons were analyzed by one-way analysis of variance (ANOVA). For the comparison of significance between groups "Tukey" was used as post hoc test. P value <0.05 was considered statistically significant (*Petrie and Sabin*, 2005).

RESULTS

Microscopic examination of H &E stained sections of the cerebral cortex from frontal area of control non- pregnant showed the external pyramidal layer showed pyramidal cells with vesicular nuclei and granule cells with vesicular nuclei (Fig.1.A.). The external pyramidal layer of pregnant showed shrunken pyramidal cells with darkly stained nuclei. Some hypertrophic pyramidal cells with faintly stained nuclei were also seen. Some granule cells with vesicular nuclei. Other granular cells appear as ghosts without nucleoli and faintly stained. Blood vessels were also seen (Fig.1.B.). The external pyramidal layer of postpartum showed normal pyramidal cells with vesicular nuclei and granule cells with vesicular nuclei (Fig.1.C.). The external pyramidal layer of *Hyoscyamus* treated mother showed granule cells with open face nuclei (Fig.1.D.). The external pyramidal layer of Anafranil treated mother, showed normal cells and dilated blood vessels (Fig.1.E.). In the fetus of pregnant group, In the fetus of pregnant group, the cerebral cortex showed normal histological features of typical layers that include ventricular zone, intermediate zone, sub plate cortical plate and marginal zone. Normal neuron and neuroglial cells were also seen (Fig.2.A.).In the postpartum offspring group showed the layers showed normal granule cells with central nuclei, normal pyramidal cells with vesicular nuclei and neuroglial cells (Fig.2.B). In the *Hyoscyamus* treated offspring, the layers showed granule cells with complete degeneration, shrunken pyramidal cells with darkly stained nuclei and cytoplasmic vacuolation (Fig.2. C.). In the Anafranil treated offspring, the layers appeared normal granular and pyramidal cells (Fig.2. D.). The morphometrical results are summarized as figures (7) & (8).

In frontal cortex of control non-pregnant, Congo red stain demonstrated small deposits of amyloid plaques (Fig.3.A).In the frontal cortex of control pregnant group, Congo red stain demonstrated severe deposits of amyloid plaques

(Fig.3.B).In the frontal cortex of postpartum mother, Congo Congo red stain demonstrated moderate deposits of amyloid plaques (Fig.3.C).In the frontal cortex of *Hyoscyamus* treated mothers, they showed degenerated amyloid plaques (Fig.3.D). In the frontal cortex of Anafranil treated mothers, they showed mild amount of amyloid plaques (Fig.3. E.).). In the frontal cortex of postpartum, *Hyoscyamus* and Anafranil treated offspring, Congo red stain demonstrated no amyloid plaques (Fig.3.F,3. G &3.H). In a general view, serotonin transporter immunoreactivity was verified predominantly in neuron and axon of the frontal cortex using anti-serotonin transporter. In the frontal cortex of control non pregnant group, showing moderate immunoreactivity in neuron and axon (fig.4.A). In the frontal cortex of control pregnant group, showing severe immunoreactivity in neuron and axon (fig.4.B). In the frontal cortex of postpartum group, showing moderate immunoreactivity in neuron and axon (fig.4.C). In the frontal cortex of *Hyoscyamus* treated mother, showing mild immunoreactivity in neuron and axon (fig.4.D). In the frontal cortex of Anafranil treated mother, showing weak immunoreactivity in neuron (fig.4.E). In the frontal cortex of postpartum, *Hyoscyamus* and Anafranil treated offspring, Immunohistochemical stain demonstrated negative immunoreactivity in neuron and axon (figs.4.F,4. G &4.H) respectively.

The morphometrical results are summarized as figures (9) & (10).

Electron Microscopic examination of cerebral cortex from frontal area of Control non- Pregnant group showed intact nucleus with normally distributed chromatin, typical mitochondria with defined cristae and normal rough endoplasmic reticulum (fig.5.A). Control Pregnant Group, frontal cortex showed typical nucleus, some degenerated mitochondria, some fragmented RER and vacuolated cytoplasm (fig.5.B). Postpartum mother, frontal cortex showed normal nucleus, normal mitochondria and normally arranged RER (fig.5.C). *Hyoscyamus* treated mother, frontal cortex showed intact nucleus, typical mitochondria with obvious cristae (fig.5.D.). Anafranil treated mother, frontal cortex showed nucleus with well distributed chromatin, degenerated mitochondria and extensive RER (fig.5.E.).

Fetus of pregnant group, the cerebral cortex showed intact nucleus with normally distributed chromatin intact nuclear membrane but degenerated mitochondria, fragmented RER and vacuolated cytoplasm (fig.6.A). Postpartum offspring's, cerebral cortex showed intact nucleus with normally distributed chromatin, intact nuclear membrane, normal mitochondria and RER stuffed with free ribosome (fig.6.B).

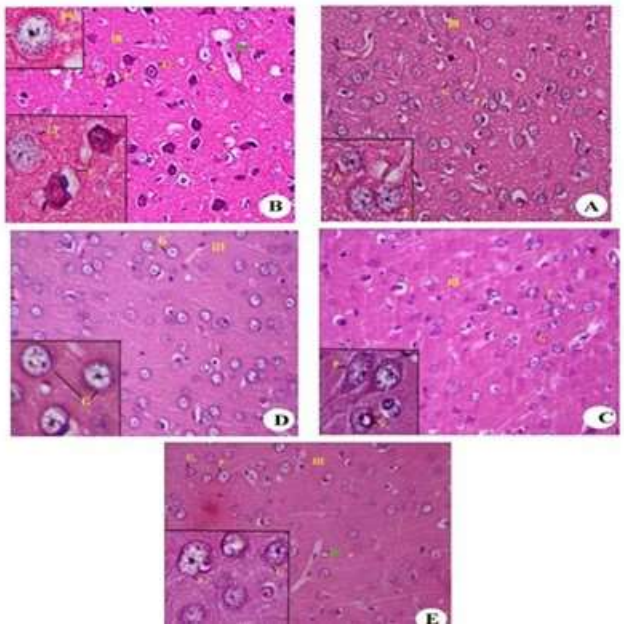
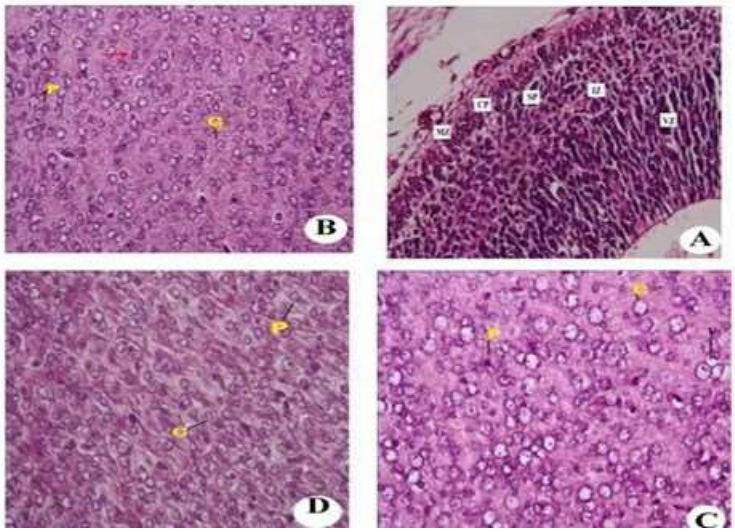


Figure1: Photomicrograph of the frontal cortex of control non pregnant (A), pregnant **(B),** postpartum **(C)**, Hyoscyamus and Anafranil treated mother **(D-E). (A)** external pyramidal (III) layer of control non pregnant group showing pyramidal cells (P) with vesicular nuclei and granule cells (G) with vesicular nuclei. (**B)** external pyramidal

(III) layer of control pregnant group showing shrunken pyramidal cells (P) with darkly stained nuclei. Some hypertrophic pyramidal cells (PY) with faintly stained nuclei were also seen. Some granule cells (G) with vesicular nuclei. Other granular cells (Gr) appear as ghosts without nucleoli and faintly stained. **Note**:-Blood vessels (BV) were also. **(C)** external pyramidal (III) layer of postpartum group showing normal pyramidal cells (P) with vesicular nuclei and granule cells (G) with vesicular nuclei.**(D)** external pyramidal (III) layer of *Hyoscyamus* treated mother group showing granule cells (G) with open face nuclei. **(E)** external pyramidal (III) layer of anafranil treated mother group showing normal pyramidal cells (P) with vesicular nuclei and granule cells (G) with vesicular nuclei. **Note**: -Blood vessels (BV) were also seen (H&E X400; inset, X1000).



Figur2: Photomicrograph of the frontal cortex of offsprings (A) frontal cortex of fetus group showing normal histological features of typical layers that include ventricular zone (VZ), intermediate zone (IZ), sub plate (SP) cortical plate (CP) and marginal zone (MZ). **Note: -** Normal neuron and neuroglial cells (X400). **(B)** frontal cortex of postpartum offspring group showing normal granule cells (G) with central nuclei, normal pyramidal cells (P) with vesicular nuclei and neuroglial cells (arrow) (X400).**C)** frontal cortex of *Hyoscyamus* treated group showing large granule cells (G) with complete degeneration. Shrunken pyramidal cells (P) with darkly stained nuclei. **Notice: -** Some cytoplasmic vacuolation (V) could be seen. **(D)** Anafranil treated group showing normal granule cells(G) with central nuclei and pyramidal cells(P) with vesicular nuclei (H&E X400).

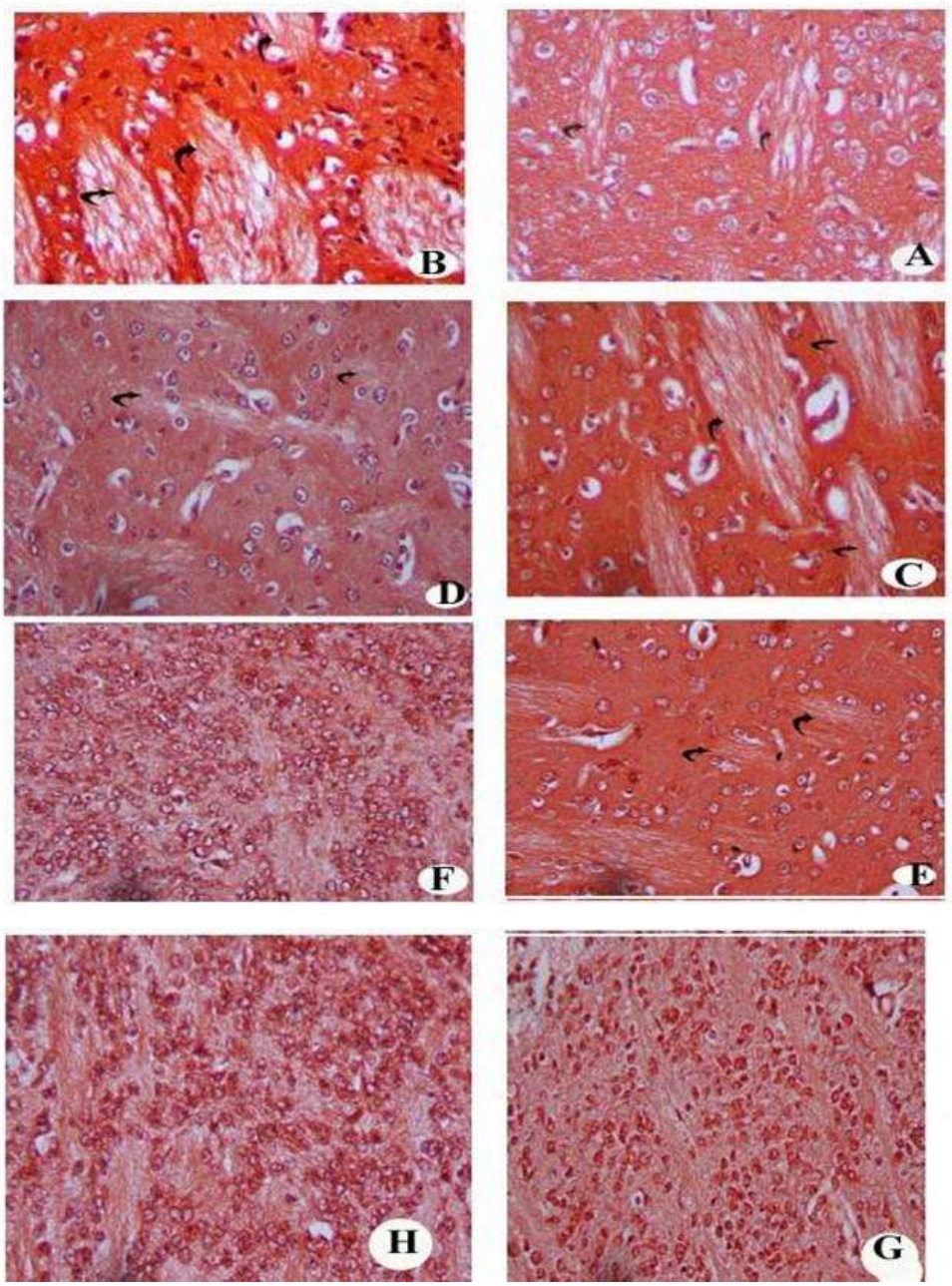


Figure3: Photomicrograph of the frontal cortex of mothers (A-E), offspring (F-H) postpartum (E-F). (A) frontal cortex of control non pregnant showing small deposits of amyloid plaques (curved arrows). (B) frontal cortex of control pregnant showing severe deposits of amyloid plaques (curved arrows). (C) frontal cortex of postpartum group showing moderate deposits of amyloid plaques (curved arrows). (D) frontal cortex of *Hyoscyamus* treated mother showing degenerated amyloid plaques (curved arrows). (E) frontal cortex of Anafranil treated mother showing mild amount of amyloid plaques (curved arrows). (F, G&H) frontal cortex of postpartum, *Hyoscyamus* and Anafranil treated offspring showing small amount of amyloid plaques (Congo red X400).

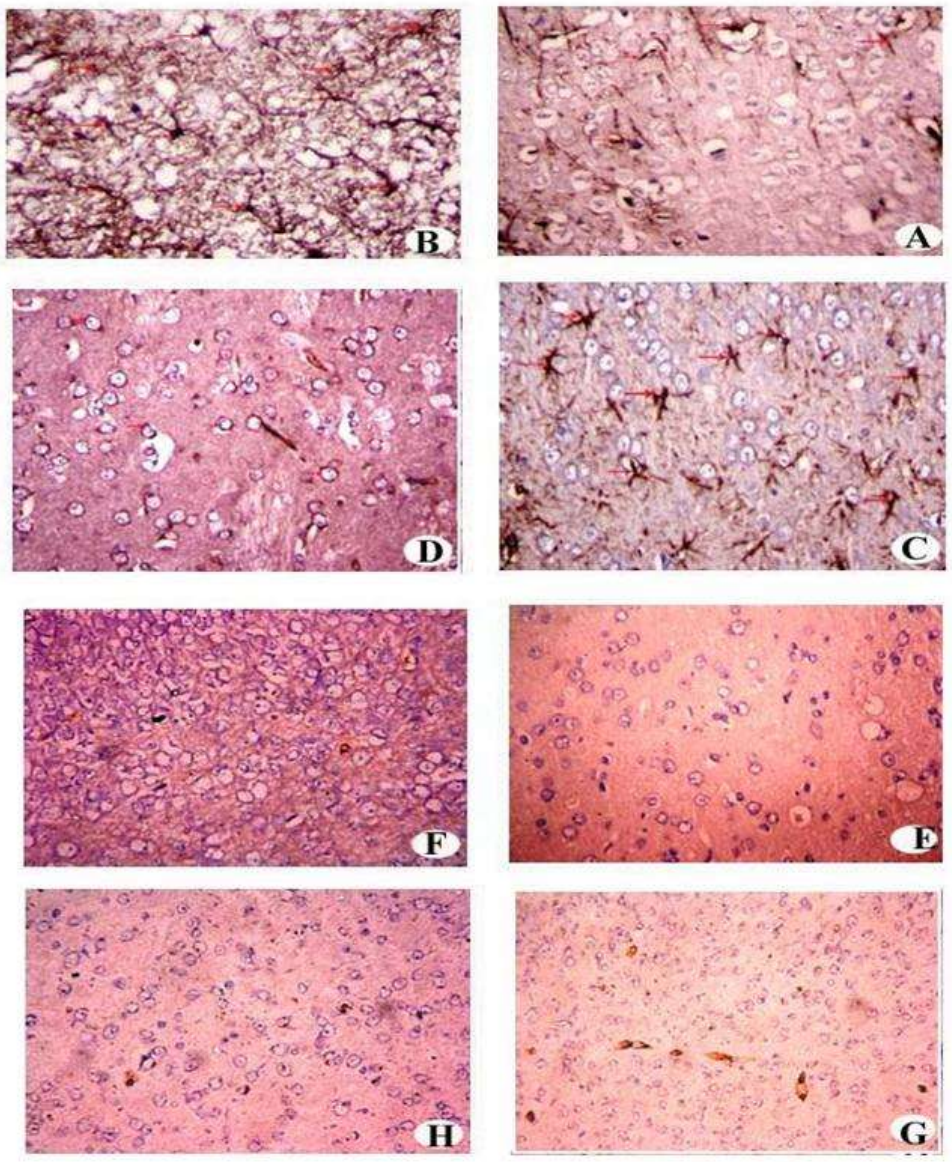


Figure4: Photomicrograph of the frontal cortex of mothers (A-E), offspring's (F-H) (A) frontal cortex of control non pregnant showing moderate immunoreactivity in neuron and axons (arrows). (B) frontal cortex of control pregnant showing severe immunoreactivity in neuron and axons (arrows). (C) frontal cortex of postpartum showing moderate immunoreactivity in neuron and axons (arrows). (D) frontal cortex of *Hyoscyamus* treated mother showing mild immunoreactivity in neuron and axons (arrows). (E) frontal cortex of Anafranil treated mother showing weak immunoreactivity in neuron (arrows). (F) postpartum offspring showing weak immunoreactivity in neuron and axon. (G&H) *Hyoscyamus* and Anafranil treated offspring showing negative immunoreactivity in neuron and axons (SERT immune X400).

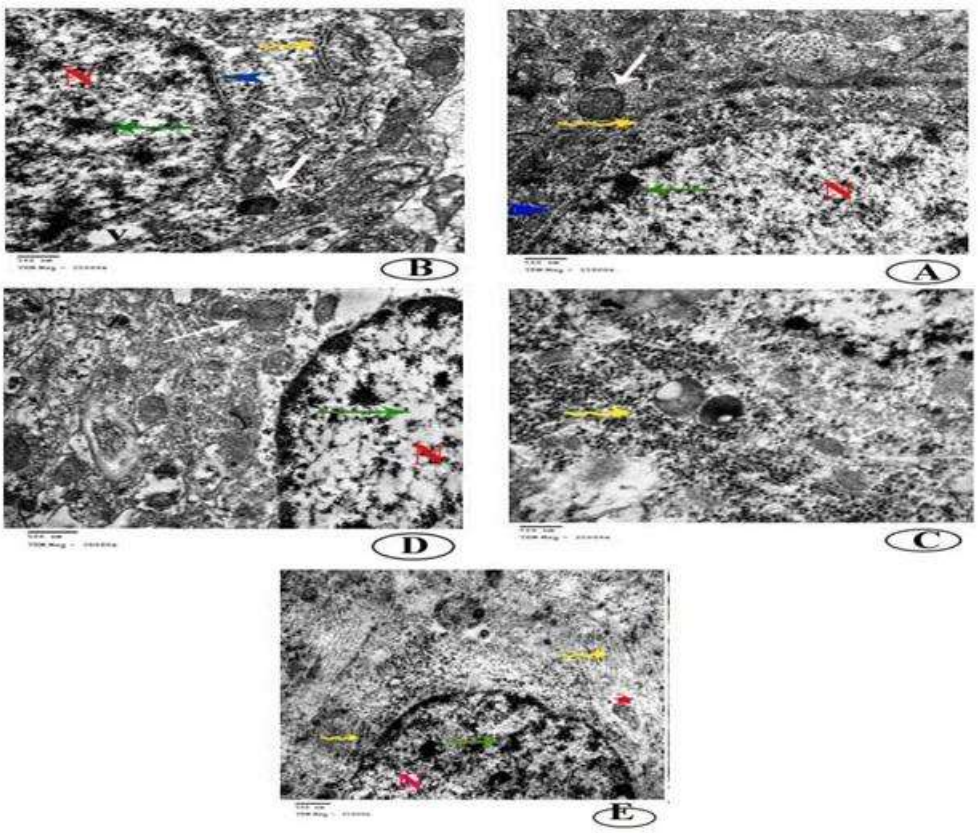


Figure 5: Electron micrograph of frontal cortex of mothers (A-E). **(A)** frontal cortex of control non pregnant group showing intact nucleus (N) with normally distributed chromatin (➞), intact nuclear membrane (➢), normal mitochondria (white arrow) with obvious cristea and normally arranged rough endoplasmic reticulum RER (∿) with free ribosomes. **(B)** frontal cortex of control pregnant group showing intact nucleus (N) with normally distributed chromatin (➞) and intact nuclear membrane (➢), some degenerated mitochondria (white arrow), normal rough endoplasmic reticulum RER (∿), some fragmented RER and vacuolated cytoplasm (V). **(C)** frontal cortex of postpartum group showing clear visible normally arranged rough endoplasmic reticulum RER (∿) with ribosomes and normal mitochondria (white arrow) with intact cristae. **(D)** frontal cortex of *Hyoscyamus* treated mother showing **nucleus** (N) with normal distributed chromatin (➞) and normal mitochondria (white arrow) with visible cristae. **(E)** frontal cortex of **Anafranil** treated mother showing nucleus (N) with well distributed chromatin (➞), normal nuclear membrane **(**➢) and normal mitochondria (white arrow) with cristae, and normally arranged rough endoplasmic reticulum RER (∿).

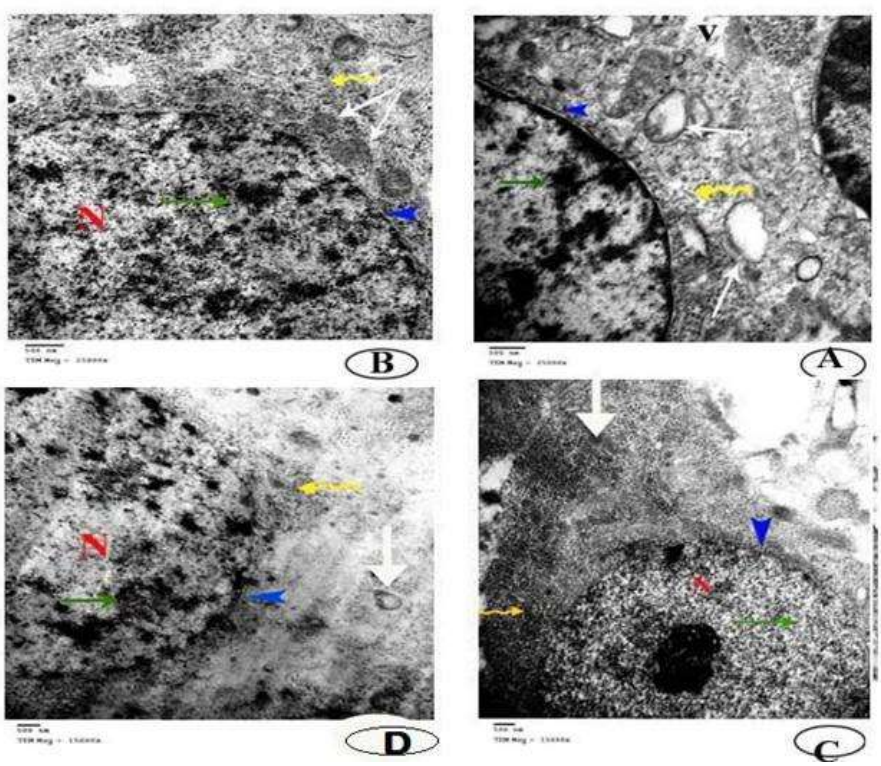
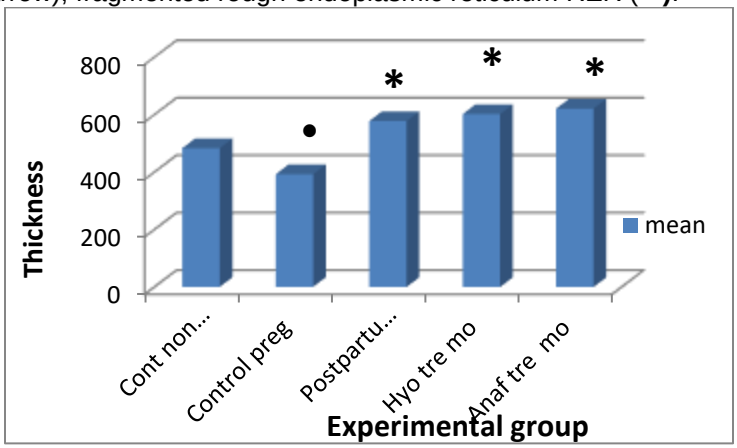
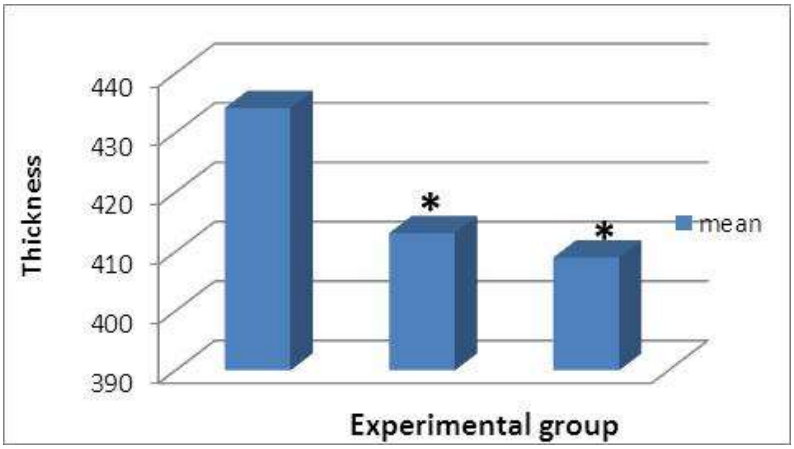


Figure 6: Photomicrograph of the frontal cortex of offspring **(A-E)**. **(A)** frontal cortex of fetus showing intact nucleus with normally distributed chromatin (➞) and intact nuclear membrane (➢), degenerated mitochondria (white arrow), fragmented rough endoplasmic reticulum RER (⇝) and vacuolated cytoplasm (V). **(B)** frontal cortex of Postpartum offspring showing intact nucleus with normally distributed chromatin (➞) and intact nuclear membrane (➢), normal mitochondria (white arrow) and rough endoplasmic reticulum RER (⇝) stuffed with free ribosome.**(C)** frontal cortex of *Hyoscyamus* treated offspring showing nucleus **(N)** with fully degenerated chromatin (➞), destructed nuclear membrane **(➢)** disintegrated mitochondria (white arrow) and fragmented rough endoplasmic reticulum RER (⇝). **(D)** frontal cortex of anafranil treated offspring showing nucleus **(N)** with fragmented chromatin (➞), degenerated mitochondria (white arrow), fragmented rough endoplasmic reticulum RER (⇝**)**.

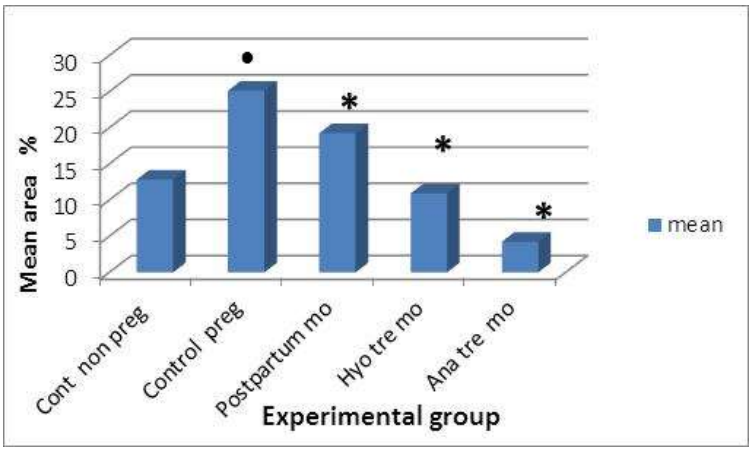


(●)= Statistically significant as compared to control non pregnant group ($p < 0.05$).
(*****)= Statistically significant as compared to control pregnant group ($p < 0.05$).
Figure 7: mean distance of frontal cortex of all experimental group and its statistically significant.



(*)= Statistically significant as compared to control pregnant group ($p < 0.05$).

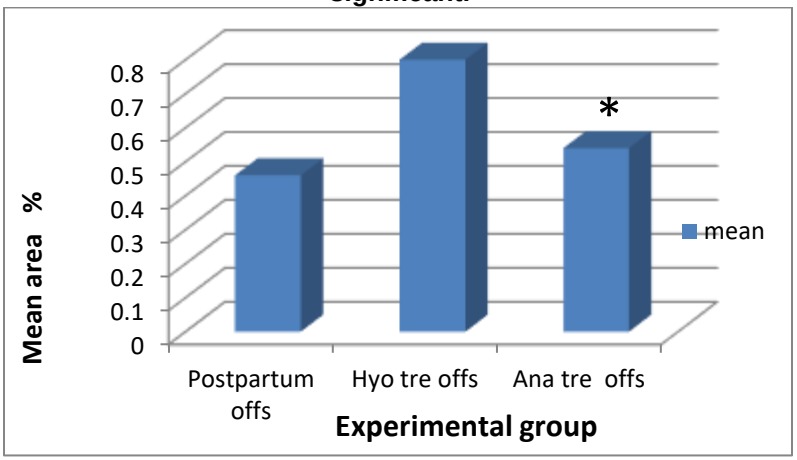
Figure 8: mean distance of frontal cortex of all experimental group and its statistically significant.



Statistically significant as compared to control non pregnant group ($p < 0.05$).

(*)= Statistically significant as compared to control pregnant group ($p < 0.05$).

Figure 9: mean area percent of frontal cortex of all experimental group and its statistically significant.



(*)= Statistically significant as compared to control pregnant group ($p < 0.05$).

Figure 10: mean area percent of frontal cortex of all experimental group and its statistically significant.

In the offspring's frontal cortex treated with *Hyoscyamus* showed nucleus (N) with fully degenerated chromatin, destructed nuclear membrane, disintegrated mitochondria and fragmented rough endoplasmic reticulum (fig.6.C.). frontal cortex treated with Anafranil treated offspring showed nucleus (N) with fragmented chromatin, degenerated mitochondria, fragmented rough endoplasmic reticulum RER (fig.6. D.).

DISCUSSION

The present study showed histological changes in the frontal cortex of pregnant rats and their offspring. The mothers were ameliorated after administration of Hyoscyamus but not antidepressant drug (Anafranil).

In pregnant women, depression has been found to be inversely associated with social support; namely women with lower social support report more depression symptoms than women with higher social support (Milgrom et al. 2019). Gestational depression is detrimental to the health of the mother and the offspring and contributes to the appearance of depressive and anxiety symptoms during the postnatal Period *Vieira et al*. (2013). Depression and other stressful feelings such as anxiety during prenatal period can be proved harmful to the mother, fetus and the expectant newborn's health. Therefore, it is essential to investigate the incidence and risk factors for antenatal depression during pregnancy (Kleanthi, 2015). These risk factors including younger age, a history of depression, exposure to domestic violence, increased life stressors, a lack of social support, unintended pregnancy, lower income, lower education, smoking, single status, and poor relationship quality (Hamel et al. 2019). The frontal cortex during pregnancy including distortion of some neuron, shrunken neurons with darkly stained nucleus, pyramidal neuron with darkly stained nuclei, and some granule cells appeared as ghosts without nucleoli and faintly stained nuclei. These results are in accordance with Oatridge et al. (2002) who detected that the reduction in brain size during pregnancy, and these results were in harmony with Hoekzema et al. (2016) who showed that grey matter volume reduces extensively across pregnancy. The changes of pregnancy could be attributed to Poromaa et al. (2017) who reported that the complex endocrine changes in women during pregnancy. Most of the endocrine changes during pregnancy are estrogens, progesterone, testosterone, prolactin, CRH, and cortisol all essentially adhere to this temporal plasma profile (Jung et al. 2011), Also, de Souza Duarte et al. (2017) said that during pregnancy, there is a large production of CRH, which is cut shortly after delivery. These changes also are in agreement with those of Liu et al. (2017) who found similar changes in prefrontal cortex during depression, including disruption and atrophy of neurons and glia. The decrease in brain size occurred with a concurrent increase in body circulatory and extracellular fluid volume and an increase in the size of other organs, such as the heart, kidney, and thyroid gland (Oatridge et al. 2002). The changes of depression could be attributed to reduction of neurotransmitters, hormonal dysregulation. This could be supported by the studies of Csaszar et al. (2014) who stated that depression is associated with decreased levels of neurotransmitters and hormonal dysregulation which were associated with macroscopic changes in the brain. Also, Oxidative stress is considered a risk factor during pregnancy and depression. This could be supported by the studies of Samir et al. (2018) who elucidated that indeed pregnancy exposes too many complications that can be related to an alteration of oxidative stress that is also associated with several pathologies during pregnancy and Vaváková et al. (2015) who stated that oxidative stress plays significant role in pathophysiology of major depression via actions of free radicals. In the present study, the decreased of blood vessels could be explained by the work of Oatridge et al. (2002) who referred to a reduction in cerebral blood flow and/or volume during pregnancy. Also, Congo Red stain showed severe deposits of amyloid plaques during pregnancy. The observation of a significantly increased deposition of amyloid plaques in the frontal cortex during pregnancy is similar to the results of Ziegler-Waldkirch et al. (2018) who said that pregnancy alone was sufficient to elevate Amyloid β plaque levels, These amyloids deposition may be a consequence of serotoninergic dysregulation and dysfunctional stress response as reported by Conejero et al. (2018). The ultrastructural features of frontal cortex during pregnancy showed mitochondrial degeneration, some fragmented RER and cytoplasmic vacuolation. It is worth noting that accumulated Aβ also contribute to mitochondrial dysfunction in AD brains (Lu et al. 2018). Mitochondrial dysfunction contributes to the pathogenesis of neurological disorders. Mitochondrial dysfunction causes increased ROS production and enhanced apoptosis, which

contribute to neurodegeneration (Zhao et al. 2019). Also, SERT immunoreactivity reactions showed severe immunoreactivity in neuron and axon during pregnancy. The observation of a significantly increased SERT in the frontal cortex during pregnancy is similar to the results of Savitz and Drevets (2013) found support for the hypothesis that functions at the SERT is increased in depression. These results could be attributed to alteration of ovarian hormones. This could be supported by the studies of (Frimer et al., 2015) who stated that the ovarian hormones have effects on key components of the serotonergic system. In Postpartum rats, there were observable improvements, in the form of increase thickness of frontal cortex, typical layers, normal neuron, decreased the amyloid deposition, they showed moderate immunoreactivity and microscopic observation showed normal mitochondria and rough endoplasmic reticulum. These changes in accordance with Oatridge et al. (2002) who found that the decrease in brain size begins after placental implantation and reversing slowly after delivery. Metabolic changes occurring during pregnancy that cause cellular atrophy may continue for some months after delivery, because metabolic substrates or nutrients initially provided for the fetus may be diverted to the neonate during lactation or to maternal tissue reparation after delivery (Oatridge et al. 2002). In Hyoscymus-treated mother, the cells appeared as normal. Congo red stain showed degenerated amyloid plaques. They showed mild immunoreactivity. Electron microscopy investigations demonstrated normal nucleus and mitochondria. These results reconcile with those of Amit Patil et al. (2013) who reported that Hyoscyamus niger possesses antidepressant like action in mouse model of depression. This may be attributed to the small concentration of scopolamine content in Hyoscyamus leaves. so, some improvements occur in hyoscyamus. (Patocka and Jelinkov, 2017) confirmed that Atropine and scopolamine differs quantitively in anti-muscarinic actions because of the difference in permeability. Scopolamine has prominent central effects at low specific doses because of its ability to cross the blood-brain barrier, while atropine lacks this ability. The decrease of SERT immunoreactivity in Hyoscyamus treated mothers similar to study of Mateo et al. (2004) who said that the interaction of cocaine with the serotonin transporter (SERT). These could be attributed to cocaine and Hyoscyamus are tropane alkaloids which inhibit SERT. In Anafranil-treated mother, normal neuron, severe dilated blood vessels and moderate amount of amyloid plaques. It showed weak immunoreactivity in neuron. The electron microscope showed normal nucleus and mitochondria. This may be due to toxic effect of benzene ring. Benzene is an important toxic material, as it is metabolized in the liver by cytochrome P450 2E1 to various phenolic metabolites which accumulate in the bone marrow. These metabolites can produce reactive free radical species. Redox cycling of these free radicals produces active oxygen that may damage cellular (El-Fiky et al. 2016). These results reconcile with Li et al.(2017) who stated that SSRIs were less effective at reducing Aβ generation in the same cellular model compared with TCAs, although a minor but significant in vivo efficacy has been observed and reported by several groups. Takano et al. (2011) who decided that Clomipramine is a tricyclic antidepressant, that act on combined serotonin and norepinephrine transporter inhibitors (SNRIs). So, it decreases the SERT immunoreactivity. In the current study, the frontal cortex of fetus and postpartum offspring showed normal histological features of typical layers. On the level of TEM, fetus showed damage in mitochondria and fragmentation of RER. Meanwhile, the postpartum offspring showed normal nucleus, mitochondria and RER. Gestational depression modifies the maternal environment and severely damages the health of the mother and the offspring (Vieira et al., 2013). In the current study, the frontal cortex of Hyoscyamus and anafranil treated offspring showed approximately more or less mischievous results as compared to control (postpartum offspring). They demonstrated negative immunoreactivity. Hyoscyamus showed large granule cells with complete degeneration, shrunken pyramidal cells with darkly stained nuclei and some cytoplasmic vacuolation. Anafranil showed normal granule cells and pyramidal cells. On the level of TEM, they showed similar deleterious effect on nucleus, mitochondria and RER. These result in accordance with Alizadeh et al. (2014) who reported that black henbane ingestion in pregnancy is not safe because atropine and other alkaloids readily pass the placenta and fetus is sensitive to tachycardia and hyperthermia. There is also sufficient evidence that all psychotropic drugs readily cross the placenta to reach the fetus and may be excreted into breast milk. Drugs in the fetus may have a higher unbound free fraction; easily penetrate into the brain (EL-Gaafarawi et al.,

2005). The deleterious results of mothers or offsprings could be attributed to the short time of recovery.

CONCLUSION

Treatment with *Hyoscyamus muticus* exerted ameliorated effects against pregnancy-induced damage on mother's frontal cortex while not affect offspring, but Anafranil showed some deletrious effects on mother and offspring.

CONFLICT OF INTEREST

The authors declared that present study was performed in absence of any conflict of interest.

AUTHOR CONTRIBUTIONS

All authors approve publication this work

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