Original Article

Effect of Induced Diabetes Mellitus on the Development and Structure of the Basolateral Amygdala Nucleus in the Offspring of Albino Rats and the Possible Protective Role of Resveratrol

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ABSTRACT

Introduction: Maternal diabetes is regarded as an important teratogen that leads to brain damage including amygdala. The basolateral nucleus of amygdala presents in temporal lobe that have important role in emotional behavior and memory. Resveratrol is a natural antioxidant.

Aim of Work: To reveal postnatal development of basolateral nucleus of amygdala. Also cytoarchitecture of basolateral nucleus in albino rat offspring of diabetic rats has been studied. In addition the possible protective role of resveratrol was evaluated.

Material and Methods: 40 female adult rats were distributed as follows I-Control group: Received no treatment, II-Diabetic group: Induction of diabetes performed through a single dose of alloxan injected in the peritoneum (150 mg/kg), III-Resveratrol treated group: Rats received resveratrol orally (20 mg/kg) daily during pregnancy and through the lactational period. Resveratrol was received in the form of powder that was dissolved in gum acacia due to its limited water solubility and IV-Resveratrol treated diabetic group: diabetic rats received resveratrol orally at the same dose as in group III. Offspring (1day "newborn", 10days and 21 days) were studied. Light microscopic, electron microscopic, morphometric, immunohistochemical, and molecular techniques (PPAR gene expression) were studied.

Results: In control group, organization of basolateral nucleus appear to be well developed in all studied groups. In the diabetic group, both light and electron microscopic studies showed degenerative changes in the neurons of the basolateral nucleus of amygdala which were confirmed by morphometric study. Immunohistochemical study revealed increased caspase-3 expression and decreased GABA concentration. Molecular studies showed decreased PPAR gamma expression. In resveratrol treated group, cells approximated those of the control group. Diabetic rats treated with resveratrol revealed obviously improved results. So, resveratrol is recommended for pregnant diabetic female to protect their children from autism.

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Key Words: Autism, basolateral nucleus, maternal diabetes, resveratrol.

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INTRODUCTION

The rat amygdala is a considerably a large mass present in the temporal lobe. It lies ventral to basal ganglia[1]. The basolateral nucleus within the amygdala is a mass of large neurons that appear densely stained in Nissl sections. Lateral to the basolateral nucleus, external capsule is found. The basolateral nucleus could be divided into two parts. The anterior magnocellular part where the larger and darker staining pyramidal neurons of the amygdala could be found. The posterior parvicellular part contains smaller pyramidal neurons with their apical dendrites aligned toward the pial surface forming compact bundles[2]. Stellate neurons that vary in size are also found. They form a heterogenous population of neurons that have smaller cell bodies than those of the pyramidal neurons. Stellate cells do not have apparent apical dendrites. Golgi stain studies revealed that stellate cells have been subdivided into multipolar, bitufted, and bipolar cells according to their dendritic trees[3]. The magnocellular part receives massive projections from the parvicellular part. The central nucleus within the amygdala receives extensive projections from the basolateral nucleus[2].

The basolateral nucleus has an important role in behavior and memory[4]. Basolateral nucleus dysfunction is linked to autism as it is one of the regions which are associated with autism spectrum disorders[5].

The basolateral nucleus contains pyramidal neurons that use glutamate and interneurons that use gamma aminobutyric acid. It was found that gamma aminobutyric acid interneurons within the basolateral nucleus of amygdala play a very important role in the inhibitory neural circuits[6]. Equilibrium between excitatory and inhibitory neurons is crucial for emotional responses modulation[7]. Some studies have reported that individuals with autism spectrum disorders (ASD) had reduced gamma aminobutyric acid interneurons in regions of the cortex and altered GABA receptor expression[8].

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As regards genetic consideration, peroxisome proliferator-activated receptor gamma (PPARγ) was found to be extensively expressed in the central nervous system[9]. Through controlling gene networks related to glucose homeostasis, PPARγ was found to affect glucose metabolism[10].

Regarding the prenatal development of the basolateral nucleus of rat amygdala, the amygdala nuclei are located at different locations within either the pallium or the subpallium. The basolateral nucleus is derived from the lateral pallium. Generally, at the 17th day of embryonic life, amygdala nuclei could be recognized. At the first day of postnatal life, amygdaloid nuclei became more distinguished[11].

Insulin receptors are widely distributed in different regions including the amygdala[12]. Diabetes mellitus causes hyperglycemia that eventually leads to tissue damage. The neurons are not dependent on insulin but they can respond to insulin changes. When neurons exhibit insulin resistance, they respond incorrectly to growth factor which eventually leads to degeneration of these neurons[13].

Neuronal dysfunction in diabetes is attributed to neuronal damage by free radicals and oxidative stress. During neurulation, the embryo is much more vulnerable to external harmful agents. Maternal diabetes leads to extensive reactive oxygen species production at the stage of neurulation with susceptible teratogenic consequences[14].

Resveratrol (RSV) is a natural phenol. Grapes, blueberries, mulberries, and peanuts are rich sources resveratrol[15]. The liver and lungs are the main sites of its metabolism[16]. Resveratrol can cross the placenta[17]. It is also excreted in milk[18]. Resveratrol is capable of crossing blood brain barrier and reaching the brain in relevant concentrations[19].

Resveratrol treatment was found to decrease the complications of diabetes mellitus, thus it may have role in treating diabetes. It was found that treatment with resveratrol also improved neurodegenerative complications of diabetes mellitus. As regards autism and its suggested treatments, resveratrol as a natural PPAR agonist could be suggested in treating autism[20].

Based on the previous data, this work aimed to study the postnatal development of basolateral nucleus of amygdala and its histological structure in albino rat offspring of diabetic rats. Additionally, the possible protective role of resveratrol was assessed.

MATERIAL AND METHODS

Chemicals and kits

1. Alloxan was obtained from Sigma company in a powder form that then used by dissolving it in a physiological saline solution (0.9% sodium chloride).
2. Resveratrol was purchased from Sigma company. It is obtained in a powder form then dissolved in gum acacia.
3. Caspase 3 was purchased from cell signaling company, cat No.9661.
4. Gamma amino butyric acid (GABA) was purchased from Bioss antibodies USA (bs-2252R).
5. Primers of PPAR gamma and GAPDH were purchased from Metabion company- Germany.
6. Total RNA Mini Extraction Kit: spin column was used in this work (catalogue number: ABT002, Applied Biotechnology-Egypt)
7. cDNA synthesis kit: (catalogue number: ABT009, Applied Biotechnology- Egypt).

Animals and diet

A total number of forty adult female rats weighing 170 to 200 grams were used in this experiment. Twenty male rats were also used for matting of female rats, five for each group. The rats were housed in well prepared cages in a room with suitable conditions at Faculty of Medicine, Assiut University. Food ad libitium and water were given to rats. The animal experimental protocol received approval from Institutional Animal Care Committee (IRB no: 17200443).

Experimental design

The 40 adult female rats were distributed into 4 groups (10 female rats for each group).

Control group: The female rats received no treatment and were kept with 5 adult male rats. The appearance of the vaginal plug is considered the first day of pregnancy[21].

Diabetic group: Induction of diabetes performed by using one dose of alloxan (150 mg/kg) injected into the peritoneum. All induced female rats showed hyperglycemia (400–600 mg/dL) two days after alloxan administration. Then the diabetic female rats were housed with 5 adult male rats. To be sure that the female rats became diabetic, the blood glucose level was measured daily via making a small puncture in the tail. The method was noninvasive and quick[22].

Resveratrol treated group: In this group, 10 female rats were kept with 5 adult male rats to become pregnant as in group I.[21]. Then each received resveratrol orally dissolved in gum acacia (20 mg / kg body weight)[23]. It was given from day one of pregnancy and continued daily during pregnancy and for 21 days after delivery[24]. The appearance of the vaginal plug is considered the first day of pregnancy[21].

Resveratrol treated diabetic group: In this group induction of diabetes performed by using one dose of alloxan (150 mg/kg) injected into the peritoneum[22]. The same technique for induction of diabetes was followed as in group II. The next step was matting between the diabetic

female rats and five adult males of the same strain. Then resveratrol given orally to diabetic rats (20 mg / kg)[23]. It was given from day one of pregnancy and continued daily during pregnancy and for 21 days after delivery[24].

The following age groups were studied

The total number of offspring was 135 offspring as follows:

1 day (newborn) (35)

10 offspring were sacrificed in each group except for group III where 5 offspring were sacrificed.

10 days old (35)

10 offspring were sacrificed in each group except for group III where 5 offspring were sacrificed.

21 days old (65)

20 offspring were sacrificed in each group except for group III where 5 offspring were sacrificed. The number of rats doubled in this age group due to the additional technique of molecular study of the PPAR gamma gene with the microscopic and morphometric study. In group III, offspring are only subjected to histological examination and morphological study.

At the end of the experiment, the offspring were sacrificed by an overdose of ether. The offspring were decapitated and the brains were extracted. For light microscopic examination the extracted brains were put in 70% ethyl alcohol. For electron microscopic examination, the extracted brains were put in a glutaraldehyde (10 %) solution.

The rat brains were processed for the following techniques

A-Light microscopic technique

Brains were prepared in blocks and cut in serial coronal sections. Gallocyanin-chrom alum stain was used to demonstrate the cytoarchitecture of the basolateral nucleus of amygdala[25].

B-Electron microscopic technique

Obtained brain specimens were fixed by using formaldehyde (10 %) solution followed by dehydration in ascending concentrations of alcohol (30, 50, 70, 90 and 100% for 120 minutes). Next step was infiltration by epoxy resin. 0.5µm thick semithin sections stained with toluidine blue. The following step was cutting ultrathin sections (80-90nm) by using ultra microtome from the area of the basolateral nucleus of amygdala[26]. Last step was to examine the stained sections and photograph them by electron microscope in the Electron Microscopic Unit in Assiut University.

C- Immunohistochemical study

Paraffin blocks of the rat brains were coronally cut at a thickness of five microns on coated slides and incubated at a temperature of 42°C for twenty four hours. Alternatively stained sections with gallocyainin were used to detect the basolateral nucleus.

Caspase-3

Polyclonal anti rabbit caspase-3 (dilution of 1: 100) was used to find neuronal apoptosis in the basolateral nucleus in amygdala. Xylene (1 hour) was used to paraffin removal from the sections. Sections then were hydrated in descending concentrations of alcohol. Next step was incubation of sections in hydrogen peroxide for approximately five minutes followed by washing in PBS (five minutes). Primary antibody adding to the sections and incubation for one and half hours was performed. Phosphate buffered saline was used for washing the sections twice (five minutes each). Secondary antibody added to the sections and incubating them again for twenty minutes, then washed using Phosphate buffered saline for three times. Then adding DAB compound to the sections then incubated for ten minutes was performed. distilled water was used to wash the sections then counterstained with Mayer's haematoxylin for two minutes. Finally, washing in a tap water, dehydration, clearing and mounting by DPX were done. All series included positive and negative controls. Tonsil specimens were used as the positive controls for caspase-3. Same steps with no primary antibody use were followed for the negative controls[27].

Gamma amino butyric acid (GABA)

The brain sections were immunohistochemically stained with rabbit antiserum of polyclonal type (dilution 1: 100). Sections were rinsed with Phosphate buffered saline(three times and 5 minutes each) and were incubated for 15 minutes with the secondary Anti-polyvalent antibody. The slides were washed (three times for 3 minutes each) then specimens were incubated with the HRP for 15 minutes and then washed three times for 3 minutes each with a wash buffer. The visualization of the reaction was carried out with Diaminobenzodiazibin DAB chromogen diluted with DAB substrate (for 10–15 minutes) until the desired staining was achieved and counterstained with Harris hematoxylin and mounted with DPX. The central and medial nuclei of the amygdala were used as positive controls as they are exclusively GABAergic. Primary antibody was replaced with phosphate buffered saline for negative controls and none of the controls revealed any GABA expression[28].

D- Morphometric study and statistical analysis

In this work cell count was performed for counting the neurons within basolateral amygdala.

Cell count of neurons was in 5 non-overlapping fields images in 5 different rats in each group. By using Image J software (version1.52, Public Domain). Cell count was performed at x400 magnification on a surface area of 71512.8 um.

Obtained data was expressed as mean and standard deviation then significance tested by one way ANOVA "Tuckey" test. Computerized statistical package SPSS software, version 13.00 was used for statistical analysis. *P value* ≤ 0.05 was reasoned as significant[29].

E-Molecular procedure and statistical analysis

Reverse transcriptase polymerase chain reaction known as RT-PCR technique was followed to measure peroxisome proliferator-activated receptor gamma (PPAR gamma) gene expression in the basolateral nucleus of amygdala of the 21 days old rats. Glyceraldehyde 3 phosphate dehydrogenase known as GAPDH was used as a reference gene. 21 days old offspring of control, diabetic and resveratrol treated diabetic rats were studied.

RNA Isolation

TRIzol solution obtained from Applied Biotechnology company was used and total RNA isolate was obtained. All RNA isolates were clean RNA isolates[30].

RT-PCR analysis

Two step semi quantitative RT-PCR procedure was followed. The first step of complementary DNA (cDNA) synthesis required the use of Oligo-(dT)18n primer. Total RNA (2 µg) combined with 2 µl oligo-dT, 100 µl dNTPs and H minus MMLV (25 µl). For denaturation the combination was heated at 95°C for five minutes. Incubated of RT mix was kept at 42°C for sixty minutes, then ceased by heating at 95°C for five minutes. The cDNA was kept at -20°C. All PCR amplifications were performed in a mixture consisting of:

For proliferator-activated receptor gamma (PPARγ) primer: 5 µl SYBR green, 0.4 µl primer forward, 0.4 µl primer reverse and 4.2 µl cDNA. For Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) primer: 7.5 µl SYBR green, 0.5 µl primer forward, 0.5 µl primer reverse and 6.5 µl cDNA.

RT-PCR performed on a MX 3000P STRATAGENE machine (The Molecular biology research unit of Assiut University). The primer sequences were as follows:

PPAR γ:	Forward: 5'-CCCTTTACCACGGTTGATTTCTC-3'. Reverse: 5'-GCAGGCTCTACTTTGATCGCACT-3'.
GAPDH:	Forward: 5'-ATGGCCTTCCGTGTTGTTCCTACCC3'. Reverse: 5'-GCCTGCTTCACCACCTTCTTGATG3'.

Electrophoresis of samples were done using agarose gels (1%) in TBE. Ethidium bromide [10 µg/ml] was used for staining. Then photographing of gels obtained using a 280 nm UV light box. A digital camera with viber lumat program used to obtain images and a DNA ladder used for evaluation[31].

Through RT-PCR, cycle threshold (CT) values were obtained for all specimens. CT values were converted into the ratio of gene expression then significance evaluation done by using one way ANOVA Tuckey test. *P value* ≤ 0.05 was reasoned to be significant[29].

RESULTS

Newborn age (One day age group)

Control (I) group: Gallocyanin stained sections of the control group showed that the organization of different amygdala nuclei could be recognized in the newborn rats. External capsule was found lateral while the central nucleus located medial to baslateral nucleus. Lateral nucleus was located dorsal and the basomedial nucleus was found ventral to the basolateral nucleus. Medial nucleus located next to optic tract (Figure 1). The basolateral nucleus contained small crowded pyramidal neurons. The nuclei were surrounded by dark basophilic cytoplasm as it is rich in nissl granules. Cell processes emerging from pyramidal neurons could be also seen in newborn age. Stellate cells with smaller cell bodies could be seen also dispersed in between the pyramidal neurons (Plate I. Figure 2). Ultrastructure of basolateral nucleus in the newborn control group showed neurons with oval nuclei and dominant nucleoli. The cytoplasm appeared rich in organelles (Plate II. Figure 6). Mitochondria and synaptic vesicles were abundant in the presynaptic terminals (Inset in Figure 6).

In the diabetic (II) group, gallocyanin stained pyramidal neurons showed nuclei with dark staining with cytoplasmic vacuolation. Stellate neurons also were affected and showed cytoplasmic vacuolation (Plate I. Figure 3). The ultrastructure of neurons showed condensed chromatin in their nuclei. The cytoplasm appeared rarified with degenerated organelles and vacuolation (Plate II. Figure 7). Higher magnification revealed that the presynaptic terminal had apparently decreased synaptic vesicles (Inset in Figure 7).

In resveratrol treated (III) group, gallocyanin staining demonstrated that the pyramidal and stellate neurons resembled those in control rat brain (Plate I. Figure 4).

In resveratrol treated diabetic (IV) group, gallocyanin stained neurons revealed that few degenerated neurons(Plate I. Figure 5).

Ultrastructure of neurons in group (IV) demonstrated that neurons had round to oval nuclei and fine granular chromatin. Dilated endoplasmic reticulum with the presence of some vacuoles could be seen (Plate II. Figure 8). Synaptic terminals showed restored synaptic vesicles (Inset in Figure 8).

Morphometric results of newborn age group

In control (I) group, the number of the neurons within basolateral nucleus per an area 71512.8 µ² was 182.24±9.75 while it was found to be 136.96±9.37 in diabetic(II) group. The decrease in number is of high significance ($P < 0.01$). In resveratrol treated (III) group, number of the neurons was 183.16 ± 8.73 which was statistically insignificantly increased contrasted with that of the group I ($P > 0.05$). In resveratrol treated diabetic (IV) group, the mean number of the neurons in the basolateral nucleus was 179.96 ± 4.61

which is statistically insignificantly decreased as contrasted with group I ($P > 0.05$). The increase in number in group IV contrasted with group II is of high significance ($P < 0.01$) (Table 1, Graph 1).

10 days old age group

In the control (I) group, gallocyanin staining showed less crowded larger pyramidal neurons with more differentiated cell processes compared to those of the newborn group. Stellate neurons also appeared larger and more differentiated when compared to those of the newborn age (Plate III. Figure 9). Electron microscopic examination of group (I) showed a pyramidal neuron contained a large round nucleus with homogenous chromatin. Numerous organelles could be seen in cytoplasm(Plate IV. Figure 13). Higher magnification revealed abundant synaptic vesicles and mitochondria in the nerve ending (Inset in Figure 13).

In the diabetic (II)group, gallocyanin stained sections of the basolateral nucleus of amygdala revealed that the pyramidal and stellate neurons showed vacuolation with dark nuclei. Other neurons showed pyknosis (Plate III. Figure 10). Ultrastructure of these neuron revealed chromatin clumping. In the cytoplasm, degenerated organelles and vacuolation could be noted (Plate IV. Figure 14). Presynaptic terminals showed apparent decreased synaptic vesicles (Inset in Figure 14).

In the resveratrol treated (III) group, gallocyanin stained pyramidal and stellate neurons appeared normal (Plate III. Figure 11).

In the resveratrol treated diabetic (IV) group, gallocyanin staining revealed that most of the pyramidal and stellate neurons have normal appearance with the presence of few affected neurons (Plate III. Figure 12). Ultrastructure of neurons of this group revealed restored organelles with residual vacuolation (Plate IV. Figure 15). Synaptic vesicles and mitochondria were also restored (Inset in Figure 15).

Morphometric results of the 10 days age group

In control (I)group, number of the neurons in the basolateral nucleus per an area 71512.8 μ^2 was 161.32±9.65 while it was found to be 113.36±8.32 in the diabetic (II) group. The decreased number was of high significance ($P < 0.01$). In resveratrol treated (III) group, the mean number was 161.96±8.50 that when compared to group I, it revealed a statistically insignificant increase ($P > 0.05$). In resveratrol treated diabetic (IV)group, number was 145.04± 27.94 that when compared to group I, the decrease was of high significance ($P < 0.01$). Comparing group IV to group II, the increase in number was highly significant ($P < 0.01$) (Table 2, Graph 1).

21 days old group

In the control (I) group, gallocyanin stained pyramidal neurons showed less crowded as compared to the age of 10 days. The pyramidal neurons have extended nerve processes while the stellate neurons were dispersed in between with smaller cell bodies (Plate V. Figure 16). Ultrastrucutral study of this group showed a pyramidal neuron with homogenous chromatin and nucleolus contained within the nucleus. The surrounding cytoplasm appeared rich in organelles (Plate VI. Figure 20). Numerous synaptic vesicles and mitochondria were seen in the synapses(Inset in Figure 20).

In the diabetic (II) group, gallocyanin staining of neurons revealed vacuolated cytoplasm and dense nuclei. Other neurons showed pyknosis. Both pyramidal and stellate neurons were affected (Plate V. Figure 17).

Electron microscopic examination of group (II) showed a neuron that has a round nucleus with a prominent nucleolus. Rarification of the cytoplasm and vacuolation were apparent (Plate VI. Figure 21). In the presynaptic terminals, apparent loss of synaptic vesicles was observed (Inset in Figure 21).

In the resveratrol treated (III) group, pyramidal and stellate neurons resembled those of the control rats (Plate V. Figure 18)

In the resveratrol treated diabetic (IV) group, light microscopic examination revealed majority of neurons appeared normal. Few affected neurons could be observed (Plate V. Figure 19). Ultrastructure of neurons in group (IV) revealed neurons that had round to oval nuclei with fine granular chromatin. Numerous organelles were seen in cytoplasm with little vacuolation (Plate VI. Figure 22). Restored synaptic vesicles and mitochondria in synapses were noted (Inset in Figure 22).

Morphometric study of the 21 days age group

In control (I) group, number of the neurons per an area 71512.8 μ^2 was 140.88±8.70 while it was found to be 81.92±8.30 in diabetic (II) group. The decrease is of high significance ($P < 0.01$). In resveratrol treated (III) group, mean number of the neurons in the basolateral nucleus was 140.96±7.72 that statistically insignificantly increased as contrasted with group I ($P > 0.05$). In resveratrol treated diabetic (IV)group, the mean number of the neurons in the basolateral nucleus was 134.60±9.64 that had statistically significant decrease in comparison with group I ($P < 0.05$). When group IV contrasted with group II, the increased neuronal number is of high significance ($P < 0.01$) (Table 3, Graph 1).

Immunohistochemical studies

Caspase 3

Caspase-3 immunohistochemical staining of basolateral amygdala in the control groups (I) of the 10 days old age (Plate VII. Figure 23) and the 21 days old age (Plate VIII. Figure 29) revealed negative to weak immunoreactivity of neurons to caspase-3.

The neurons in the diabetic groups (II) of the 10 days old age (Plate VII. Figure 24) and the 21 days old age (Plate VIII. Figure 30) revealed strong caspase-3

immunoreactivity that appeared as dark brown color of many neurons.

In the resveratrol treated diabetic groups (VI) of the 10 days old age (Plate VII. Figure 25) and the 21 days old age (Plate VIII. Figure 31), the neurons revealed mild caspase-3 immunoreactivity that appeared as light brown color of few neurons.

(GABA)

GABA immunohistochemical staining of basolateral amygdala of the control groups (I) in the 10 days old age (Plate VII. Figure 26) and the 21 days old age (Plate VIII. Figure 32) revealed strong positive GABA immunoreactivity of neurons that were presented with their dark brown color. The GABA immunohistochemical reaction appeared in the cytoplasm as a rim of brown color around the nucleus and within the processes.

The basolateral amygdala of the diabetic groups (II) of the 10 days old age (Plate VII. Figure 27) and the 21 days old age (Plate VIII. Figure 33) exhibited weak expression of GABA that could be represented by light brown color of the neurons.

In the resveratrol treated diabetic groups (VI) of the 10 days old age (Plate VII. Figure 28) and the 21 days old age (Plate VIII. Figure 34), neurons revealed that they restored their content of GABA. They appeared to have dark brown color in the cytoplasm and processes.

Molecular studies

Gel electrophoresis on agarose gels (1%) was performed for the PCR samples. DNA ladder was used to estimate the base pair size of DNA fragments for PPARγ (Figure 35) and for GAPDH (Figure 36).

Statistical analysis revealed that in the 21 day aged rats of the control lactating mothers relative gene expression of PPAR gamma in the amygdala was 1.2222± 0.66291 while it was found to be .0053 ± .00287 in the 21 day aged rats of the diabetic lactating mothers. The decrease is of high significance ($P < 0.01$).

In the 21 day aged rats of the diabetic lactating mothers treated with resveratrol, relative gene expression in the amygdala was 1.0478 ± 0.63820 with statistically insignificant decrease in the mean value as contrasted with the control ($P > 0.05$). While relative gene expression in this group showed an increase with high significance when contrasted with diabetic group ($P < 0.01$) (Table 4,Graph 2).

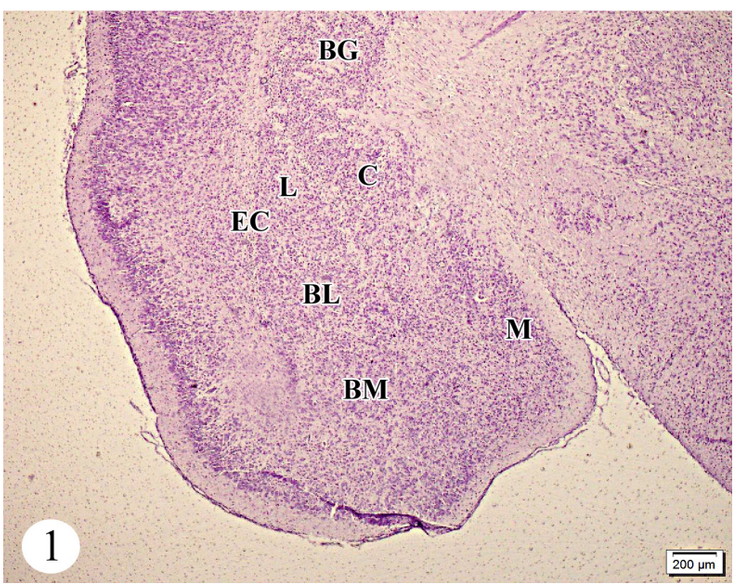


Fig. 1: A photomicrograph of coronal section of control newborn rat brain showing the main nuclei of the amygdaloid complex within the temporal lobe: Central nucleus(C), medial nucleus(M), lateral nucleus(L), basolateral nucleus(BL) and basomedial nucleus(BM). The basal ganglia(BG) are located dorsal to the amygdala. External capsule(EC) is noticed. Gallocyanin x40.

Plate.I

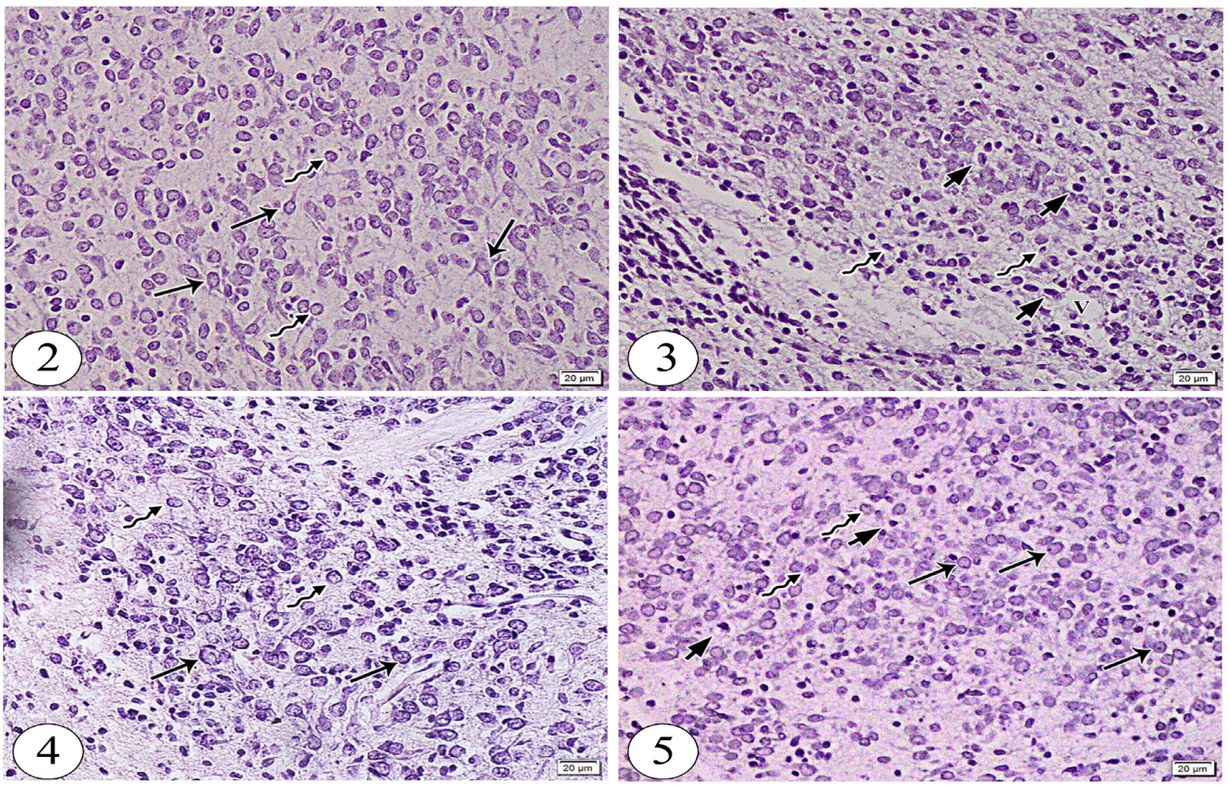


Plate I: Coronal sections stained with gallocyanin of brains in newborn rats showing the basolateral nucleus of amygdala (magnification x400). **Fig. (2):** In a newborn control rat, pyramidal neurons appear small crowded with small rounded vesicular nuclei(arrows). Axons can be noticed emerging from the pyramidal neuron. Stellate cells appear also among the pyramidal neurons with characterizing smaller cell bodies and lacking the apical dendrites (wavy arrows) **Fig. (3):** In a newborn rat of diabetic mother, pyramidal neurons have nuclei with dark staining and cytoplasmic vacuolation(short arrows). Stellate cells also appear with vacuolated cytoplasm and dark nuclei (wavy arrows). Notice the presence of vacuolations(V) in the neuropil. **Fig. (4):** In a newborn rat of resveratrol treated mother, pyramidal neurons appear small crowded that contain nuclei and prominent nucleoli(arrows) which surrounded by darkly stained cytoplasm. Note the presence of stellate cells with smaller cell bodies (wavy arrows) **Fig. (5):** In a newborn rat of resveratrol treated diabetic mother, most of pyramidal neurons appeared to have normal appearance(arrows). Few neurons contained darkly stained nuclei with cytoplasmic vacuolation(short arrows). Most of stellate cells also show improvement (wavy arrows).

Plate.II

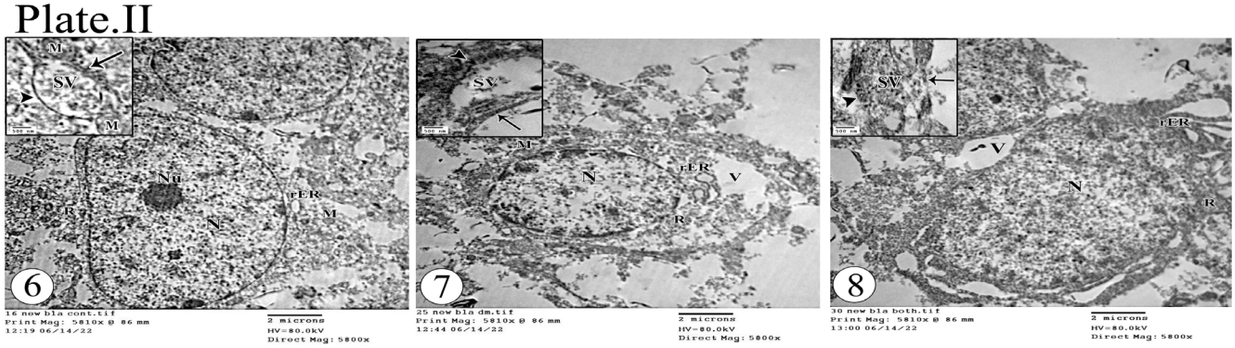


Plate II: Ultrastructure of pyramidal neurons within the basolateral nucleus in amygdala of newborn rats. **Fig. (6):** In a control newborn rat, the neuron contains large oval nucleus(N) with dominant nucleolus(Nu). The surrounding cytoplasm shows free ribosomes (R), mitochondria(M), and rough endoplasmic reticulum(rER). (TEM, x5800). (Inset): Higher magnification reveals synapses with numerous mitochondria(M) and synaptic vesicles(SV) in the presynaptic terminal(arrow head) that makes synaptic junction with the neurons of the basolateral nucleus(arrow). (TEM, x14000). **Fig. (7):** In a newborn rat of a diabetic mother, neurons appear with nucleus(N) showing condensed chromatin. The surrounding cytoplasm appears rarified and shows vacuolation(V). Notice the presence of few free ribosomes(R), dilated rER and mitochondria with damaged cristae(M). (TEM, x5800). (Inset): Shows diminished amount of synaptic vesicles(SV) in the presynaptic terminals(arrow head) making synaptic contact (arrows) on the neurons as compared with the control. (TEM, x14000). **Fig. (8):** In a newborn rat of resveratrol treated diabetic mother, neurons exhibited round to oval nuclei(N) that contain homogenous chromatin. free ribosomes(R) and dilated rER were abundant in the cytoplasm. Notice the presence of some vacuoles(V). (TEM, x5800). (Inset): showing synaptic contact(arrows) with restored synaptic vesicles(SV) in the presynaptic terminals(arrow heads). (TEM, x14000).

Plate.III

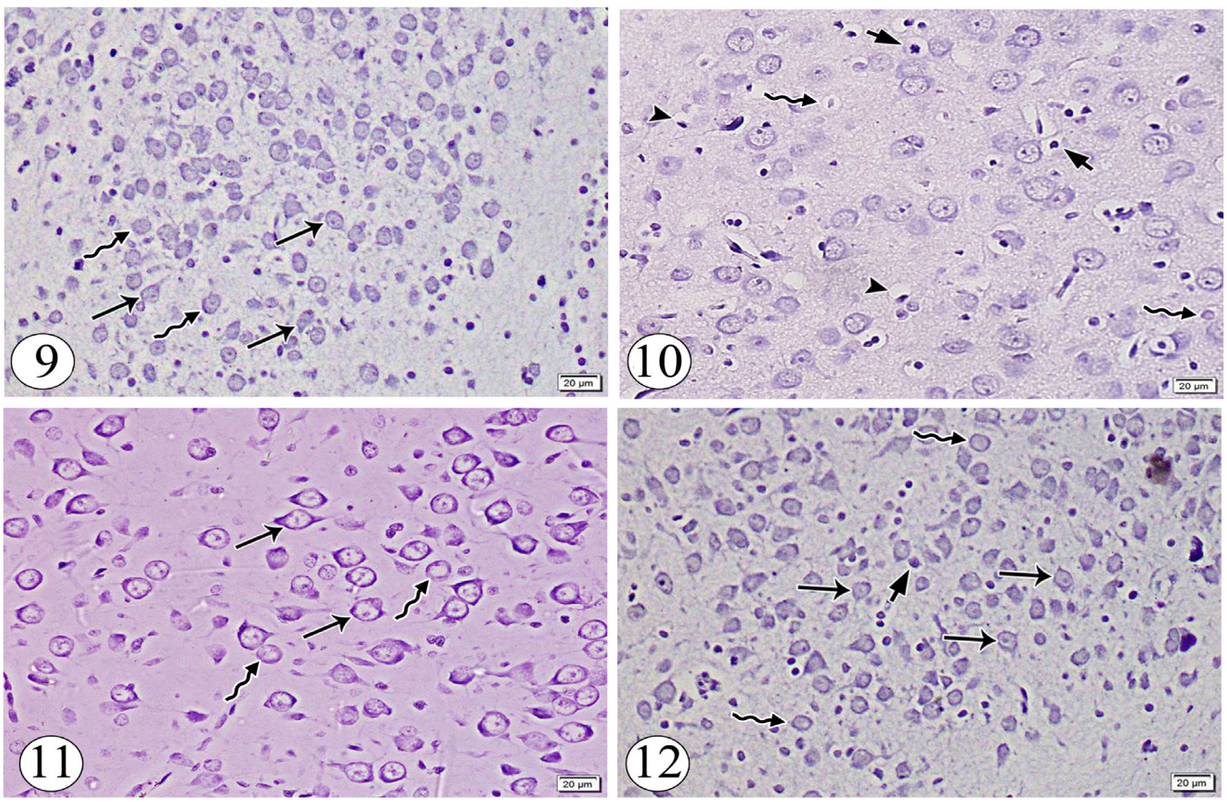


Plate III: Coronal sections stained with gallocyanin of brains in 10 days old rats showing the basolateral nucleus of amygdala (magnification x400). **Fig. (9):** In a control 10 days old rat, pyramidal neurons have vesicular nuclei and prominent nucleoli(arrows). Neurons appear larger in size and less crowded in comparison with those of the newborn group. Note the presence of many nerve processes emerging from the principle neuron. Stellate cells are seen having smaller cell bodies with no prominent axons (wavy arrows). **Fig. (10):** A rat aged 10 days of diabetic lactating mother shows many pyramidal neurons with cytoplasmic vacuolation and dark nuclei(short arrows). Note some neurons that show pyknosis(arrow heads). Stellate cells also showed vacuolated cytoplasm (wavy arrows) **Fig. (11):** In a rat aged 10 days of resveratrol treated lactating mother, pyramidal neurons contain vesicular nuclei with notable nucleoli(arrows). Many nerve processes emerging from pyramidal neurons can be noted. Stellate cells also are seen (wavy arrows) **Fig (12):** In a rat aged 10 days of resveratrol treated diabetic lactating mother shows that most of pyramidal neurons have large vesicular nuclei(arrows). Few neurons appear exhibit vacuolation and dense nuclei(short arrow). Improved stellate cells are also seen (wavy arrows).

Plate.IV

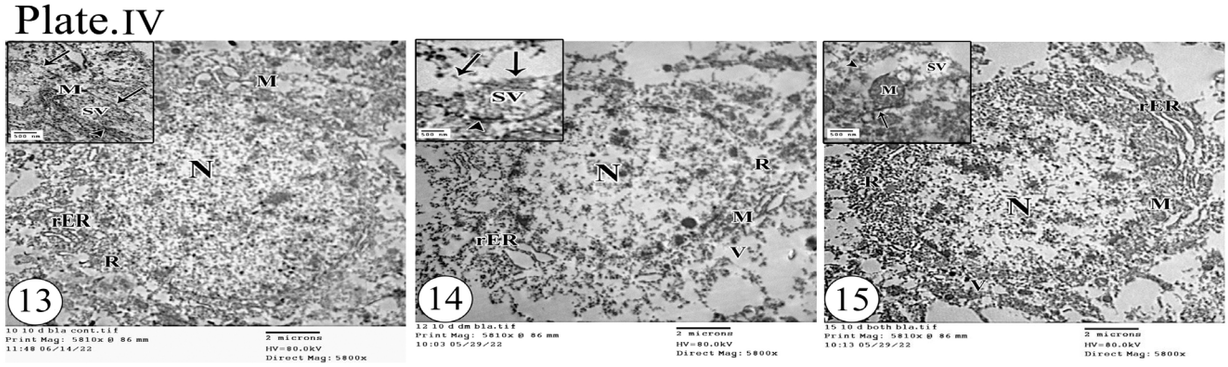


Plate IV: Ultrastructure of pyramidal neurons within the basolateral nucleus in amygdala of 10 days old rats. **Fig. (13):** Showing neurons in amygdala of 10 days old control rat. The neuron contains a large round nucleus(N) with homogenous chromatin. Abundant mitochondria (M), rER and free ribosomes(R) can be seen. (TEM, x5800). (Inset): showing many synaptic junctions(arrows) between the presynaptic nerve endings(arrow heads) and neurons. In addition to numerous synaptic vesicles(SV), abundant mitochondria(M) is present. Part of the nucleus(N) of neuron is seen. (TEM, x14000). **Fig. (14):** In a rat aged 10 days of diabetic lactating mother. It shows a nucleus(N) with chromatin clumping. Rarified cytoplasm shows damaged mitochondria(M), few free ribosomes(R), dilated rER and vacuolation(V). (TEM, x5800). (Inset): Apparently decreased amount of synaptic vesicles(SV) within presynaptic terminal(arrow heads) making contact (arrows) with the neurons can be noticed. (TEM, x14000). **Fig. (15):** In a rat aged 10 days of resveratrol treated diabetic lactating mother, A neuron contains round nucleus(N). Mitochondria(M), abundant ribosomes(R) and mildly dilated rER are seen in cytoplasm. Some vacuoles(V) are also seen. (TEM, x5800). (Inset): Showing restored synaptic vesicles(SV) and mitochondria(M) within presynaptic terminal(arrow head) making contact(arrow) with the neurons. (TEM, x14000).

Plate.V

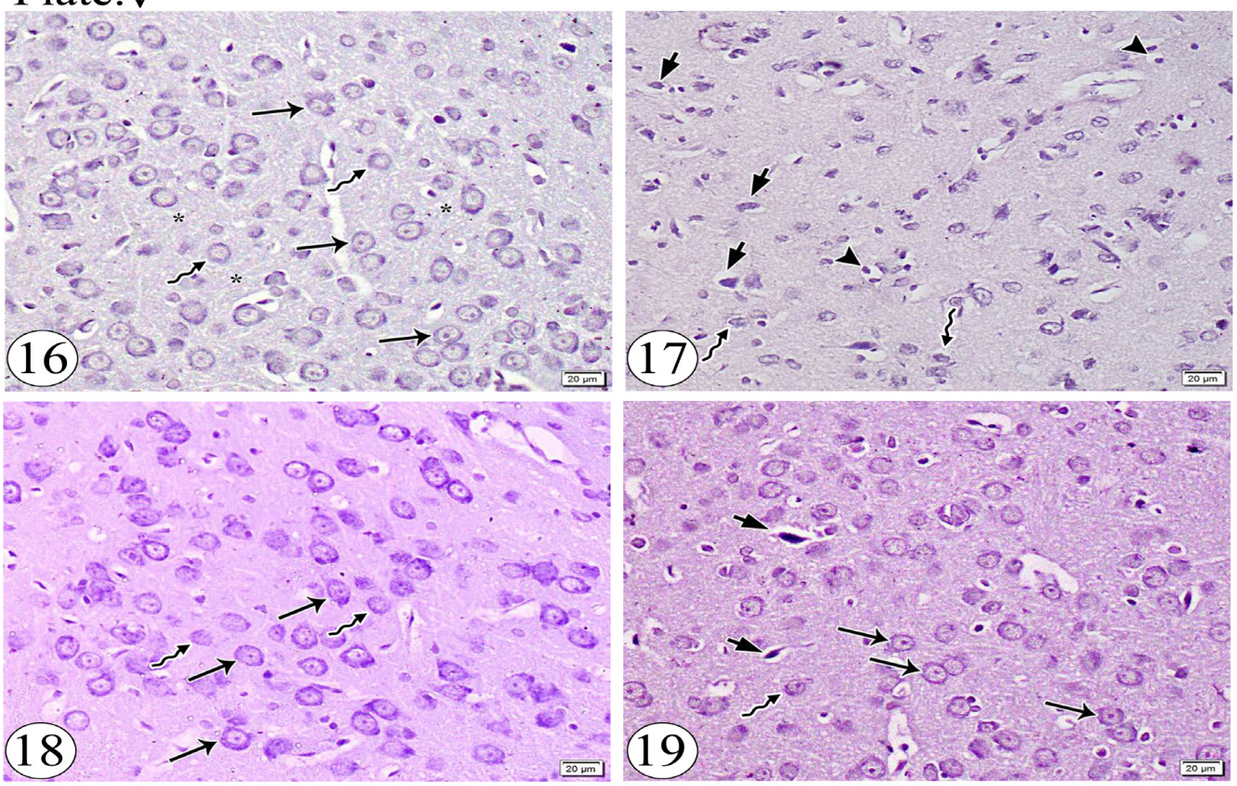


Plate V: Coronal sections stained with gallocyanin of brains in 21 days old rats showing the basolateral nucleus of amygdala (magnification x400).**Fig. (16):** In a control rat aged 21 days, pyramidal cells contain large vesicular nuclei with clearly seen nucleoli(arrows). They have darkly stained cytoplasm. Less crowdness of the neurons is noticed as compared to the 10 days group. Note that the pyramidal neurons have extended nerve processes(stars). Stellate neurons with no apical dendrites are also noted (wavy arrows) **Fig. (17):** In a rat aged 21 days of diabetic lactating mother, cytoplasm within some pyramidal neurons shows vacuolation with dense nuclei(short arrows). Other neurons show pyknosis(arrow heads). Affected stellate neurons with vacuolated cytoplasm are also seen (wavy arrows) **Fig (18):** In a rat aged 21 days of resveratrol treated lactating mother, pyramidal neurons have nuclei with vesicular appearance and dominant nucleoli(arrows). Stellate neurons with no prominent axons are also noted (wavy arrows) **Fig. (19):** In a rat aged 21 days of resveratrol treated diabetic lactating mother, most of pyramidal neurons have large vesicular nuclei(arrows). Few cells show dense nuclei(short arrows). Stellate cells also show improvement (wavy arrow).

Plate.VI

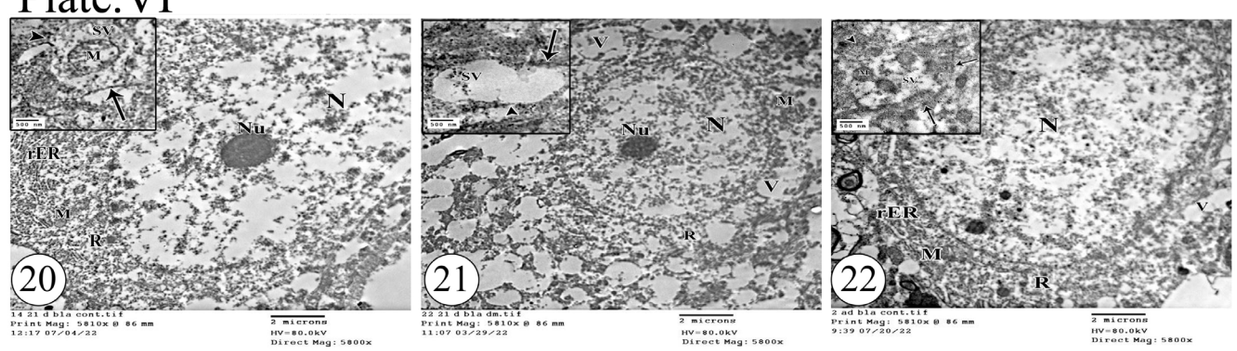


Plate VI: Ultrastructure of pyramidal neurons within the basolateral nucleus in amygdala of 21 days old rats. **Fig. (20):** In control group, a neuron contains rounded nucleus(N) with homogenous chromatin with an obvious nucleolus(Nu). Abundant free ribosomes(R), mitochondria(M) and rER fill the cytoplasm. (TEM, x5800). (Inset): A synapse shows the presynaptic terminal(arrow head) making contact(arrow) with the neuron has numerous mitochondria(M) and synaptic vesicles(SV). (TEM, x14000). **Fig. (21):** In diabetic group, a neuron have rarified cytoplasm with many vacuoles(V), few ribosomes(R) and damaged mitochondria(M). Nucleolus(Nu) is seen within the nucleus(N). (TEM, x5800). (Inset): Synaptic vesicles(SV) are obviously diminished in the presynaptic terminals(arrow heads) making junction(arrows) with the neuron. (TEM, x14000). **Fig. (22):** In resveratrol treated diabetic group, a neuron contains nucleus(N) with finely distributed chromatin. Cytoplasm shows increased mitochondria(M), free ribosomes(R) and rER with residual vacuolation(V). (TEM, x5800). (Inset): synapses appear rich in synaptic vesicles(SV) and mitochondria (M) in the presynaptic terminals(arrow heads) making contact with the neurons(arrows). (TEM, x14000).

Plate.VII

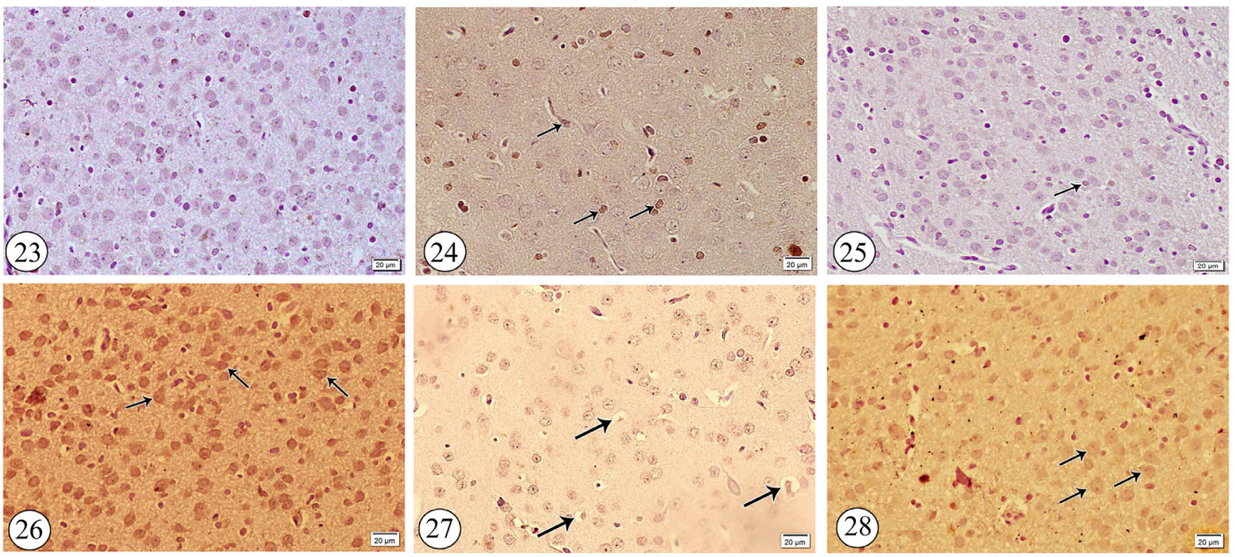


Plate VII: Immunohistochemical staining of coronal sections of 10 days old rat brains demonstrating basolateral nucleus of amygdala. **Fig. (23):** The control group reveals negative caspase-3 immunohistochemical staining. **Fig. (24):** In diabetic group, neurons exhibit strong positive expression of caspase-3 that is represented by their dark brown color (arrows). **Fig. (25):** In resveratrol treated diabetic group, diminished expression of caspase-3 is represented by light brown color(arrow) of few neurons. Caspase-3 immunohistochemical staining. x400. **Fig.(26):** GABA immunohistochemical staining of a control rat brain reveals strong positive GABA expression of the neurons which represented by dense brown staining(arrows). **Fig.(27):** In diabetic group, GABA is weakly expressed in neurons represented by their light brown color(arrows). **Fig.(28):** In resveratrol treated diabetic group, the neurons show moderate expression of GABA compared to the diabetic group(arrows). GABA immunohistochemical staining x400.

Plate.VIII

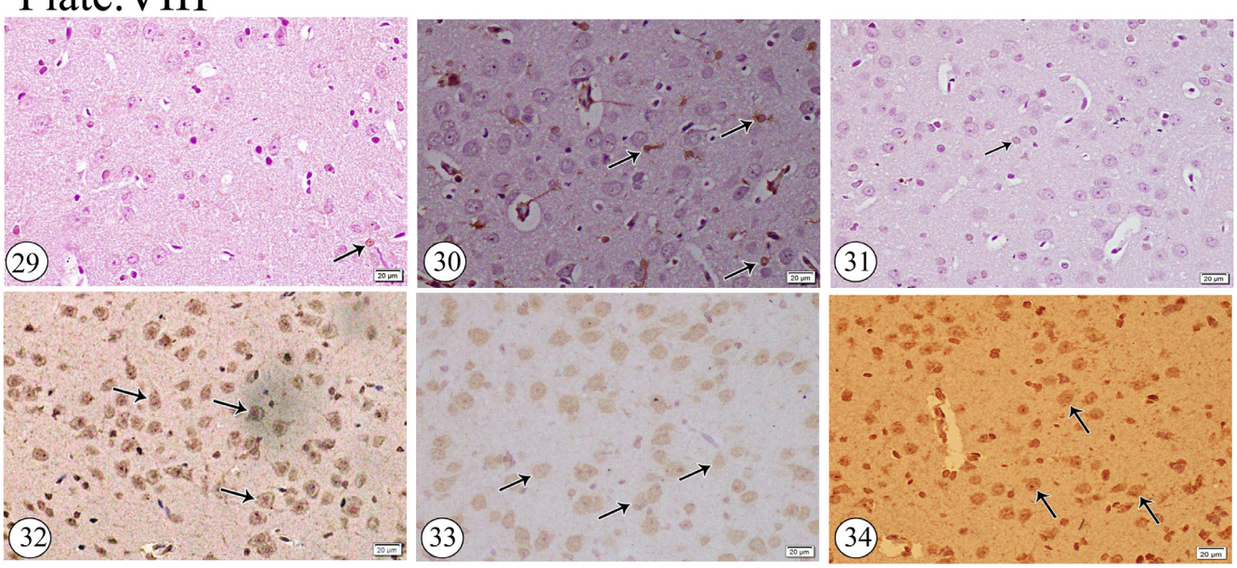


Plate VIII: Immunohistochemical staining of coronal sections of 21 days old rat brains showing basolateral nucleus of amygdala. **Fig. (29):** In a control rat, neurons show weak reaction of caspase-3. Weak immunoreactivity is represented by light brown staining of few neurons(arrow). **Fig. (30):** Diabetic group shows strong immunoreactivity of neurons to caspase-3 represented by dark brown color(arrows). **Fig. (31):** In resveratrol treated diabetic group, neurons exhibit weak immunoreactivity to caspase-3 with light brown color of the cytoplasm (arrow). Caspase-3 immunohistochemical staining. x400. **Fig.(32):** In a control rat, neurons show cytoplasmic deep brown staining and also in their processes indicating strong expression of GABA(arrows). **Fig.(33):** In diabetic group, neurons show weak expression of GABA(arrows). **Fig.(34):** In resveratrol treated diabetic group, restored GABA expression indicated by dark brown color of the cytoplasm of the neurons and their processes(arrows). GABA immunohistochemical staining x400.

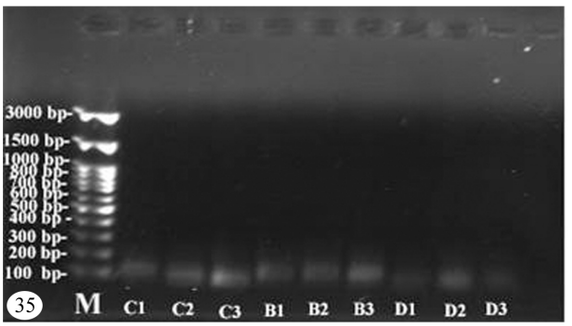


Fig. 35: Agarose gel electrophoresis (1 %) of PPAR gamma in the amygdala of 21 days old rats. PPAR gamma of the control rats (Lanes C1, C2, C3). PPAR gamma of resveratrol treated diabetic mothers (Lanes B1, B2, B3). PPAR gamma of the diabetic mothers (Lanes D1, D2, D3). Lane M refers to DNA size marker (100 bp DNA ladder H3 RTU).

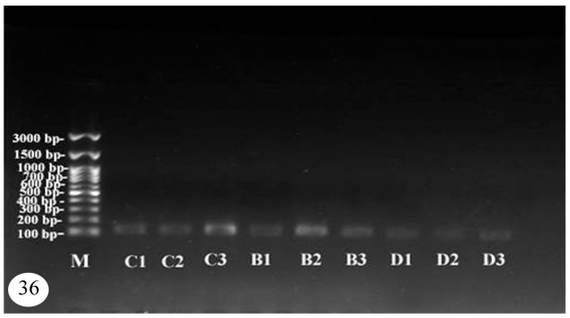


Fig. 36: Agarose gel electrophoresis (1 %) of GAPDH in the amygdala of 21 days old rats of the 3 studied groups. GAPDH of the control rats (Lanes C1, C2, C3). GAPDH of the resveratrol treated diabetic mothers (Lanes B1, B2, B3). GAPDH of the diabetic mothers (Lanes D1, D2, D3). Lane M refers to DNA size marker (100 bp DNA ladder H3 RTU).

Table 1: The mean value of cell count of neurons of the basolateral nucleus of amygdala ± SD (per an area 71512.8 μ²) of control, diabetic (DM), resveratrol treated and resveratrol treated diabetic groups in the newborn albino rats.

BLA	Control (Group I)	DM (Group II)	Resveratrol treated (Group III)	Resveratrol treated diabetic (Group IV)
Mean ± SD	182.24 ± 7.95	136.96 ± 9.37	8.73 ± 183.16	4.61 ± 179.96
P-value[1]		0.000*	0.681	0.309
P-value[2]			0.000*	0.000*

P value 1 compares the three experimental groups to the control group.
P value 2 compares resveratrol and resveratrol treated diabetic groups to diabetic group.

Table 2: The mean value of cell count of neurons of the basolateral nucleus of amygdala ± SD (per an area 71512.8 μ²) of control, diabetic (DM), resveratrol treated and resveratrol treated diabetic groups in the 10 day old albino rats.

BLA	Control (Group I)	DM (Group II)	Resveratrol treated (Group III)	Resveratrol treated diabetic (Group IV)
Mean ± SD	161.32 ± 9.65	113.36 ± 8.32	161.96 ± 8.50	145.04 ± 27.94
P-value[1]		0.000*	0.887	0.000*
P-value[2]			0.000*	0.000*

P value 1 compares the three experimental groups to the control group.
P value 2 compares resveratrol and resveratrol treated diabetic groups to diabetic group.

Table 3: The mean value of cell count of neurons of the basolateral nucleus of amygdala ± SD (per an area 71512.8 μ²) of control, diabetic (DM), resveratrol treated and resveratrol treated diabetic groups in the 21 day old albino rats.

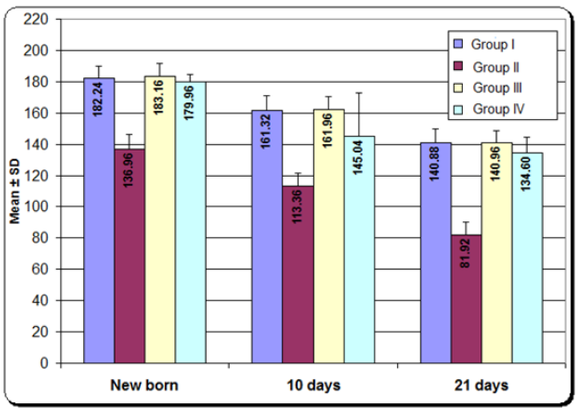
BLA	Control (Group I)	DM (Group II)	Resveratrol treated (Group III)	Resveratrol treated diabetic (Group IV)
Mean ± SD	140.88 ± 8.70	81.92 ± 8.30	140.96 ± 7.72	134.60 ± 9.64
P-value[1]		0.000*	0.974	0.012*
P-value[2]			0.000*	0.000*

Using one-way ANOVA:
P value 1 compares the three experimental groups to the control group.
P value 2 compares resveratrol and resveratrol treated diabetic groups to diabetic group.

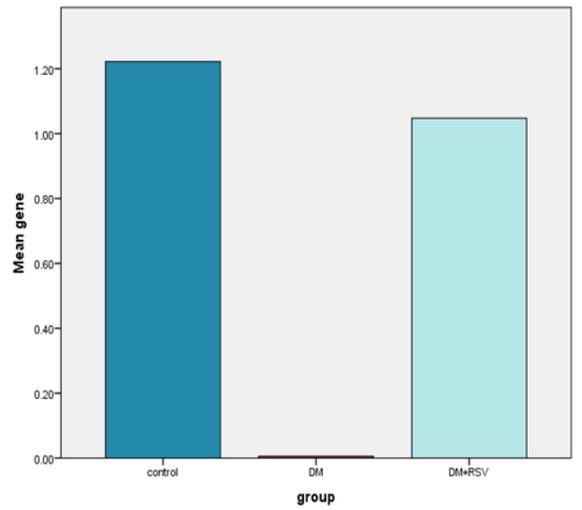
Table 4: The mean value of of gene expression of control, diabetic (DM), and resveratrol treated diabetic groups (DM+RSV) in the amygdala of the 21 days albino rats.

Gene expression	Control	DM	DM+RSV
Mean ± SD	66291. 1.2222±	00287. ± 0053.	1.0478 ± .63820
P-value[1]		0.000*	768.
P-value[2]			001.

Using one-way ANOVA:
P value 1 compares the experimental groups to the control group.
P value 2 compares resveratrol treated diabetic groups to diabetic group.



Graph 1: Showing the variation of cell count in the basolateral nucleus (per an area 71512.8 μ²) of the studied groups in different developmental ages of albino rats.



Graph 2: Showing the variation of PPAR gamma gene expression in the basolateral nucleus of amygdala studied groups of the 21 days old rats.

DISCUSSION

In the present work, the amygdala development and structure were studied. The basolateral nucleus is involved in many neural circuits which has important role in the emotional behavior and memory

Light microscopic examination of the control groups of different developmental age groups revealed the organization of the amygdala nuclei. Pyramidal neurons within the basolateral nucleus in newborn age group appeared crowded and small in size. The neurons became less crowded and larger in size at the older studied ages. This is supported by previous studies[32].

The neurons within the basolateral nucleus appeared without laminar organization and the dendrites appeared to spread in different directions. Stellate cells also were present among the principle pyramidal neurons with smaller cell bodies. These results were supported by previous studies[4].

Ultrastructural study of the basolateral nucleus of the developmental control groups revealed numerous free ribosomes in the cytoplasm of the neurons of the newborn age. With increasing age, the ribosomes gradually became on the membranes of the rough endoplasmic reticulum. The cytoplasm gradually exhibited an increase in the rough endoplasmic cisternae and mitochondria. Previous research also reported similar results in the pyramidal neurons of the cerebral cortex in rats and explained that the plenty of free ribosomes was needed for an increased internal protein synthesis of these rapidly growing neurons. Thereafter, increased rough endoplasmic reticulum indicated increased protein synthesis for external demands[33].

The morphometric studies in the developmental control groups revealed that the cell count in basolateral nucleus was decreased during the process of development in the offspring. This could be explained by process of apoptosis[34]. Apoptosis represents a regressive event that occur during brain development[35]. Axon pruning is also reported as another regressive process that occurs during the nervous system development for removal of unneeded connections[36].

In this work, the caspase-3 immunohistochemical staining of the control groups exhibited few light brown stained neurons. At younger ages, few apoptotic cells were accepted. This came in line with previous research that reported the presence of few apoptotic cells in the rat amygdala was accepted till the age of 15 day postnatally. This could be explained by that caspase-3 shared in the pathways needed for natural death of neurons at this duration[34].

GABA immunohistochemical staining of the basolateral nucleus in the control groups revealed dark brown cytoplasm of neurons and their dendrites that represented their strong positive expression of GABA[37].

The development of the GABA producing neurons within basolateral amygdala occurs between 14th and 30th days of postnatal development. It requires the appearance of parvalbumin interneurons. They are variety of interneurons that represent about forty percent of GABA interneurons[6]. Previous research also stated that the percentage of GABA interneurons in the basolateral nucleus of amygdala varied along the anterior-posterior levels[38].

Maternal diabetes effect on the development and structure of the basolateral nucleus of amygdala in the offspring was studied as it is a common metabolic disorder. The frequency of congenital malformations of the nervous system was reported to be higher in offspring of diabetic mothers[14].

Diabetes mellitus leads to hyperglycemia that eventually causes damage of various tissues particularly the nervous system. In humans, stable blood glucose level is necessary for the neuronal function. Insulin receptors were found widely spread in the different brain regions including the amygdala[12].

Current study revealed that the gestational diabetes resulted in degenerative changes in the neurons of the basolateral nucleus. Light microscopic examination revealed that the degenerative changes affected both pyramidal neurons and stellate cells. These changes were apparent from the gestational day1. The ultrastructure of the pyramidal neurons in the offspring of diabetic mothers revealed damaged organelles and vacuolations. A previous research supported these results[39].

In this work, the presynaptic terminals in the basolateral nucleus of the diabetic group showed degeneration. Alteration of synapses formation is thought to be a mechanism through which maternal diabetes could affect the brain development[40].

Morphometric studies revealed that maternal diabetes caused significant decrease in the cell number in all studied ages as compared to the control. This confirmed the previous histologic studies. Several investigations reported similar results in different brain regions[39,41].

In the present work, immunohistochemical study using Caspase-3 revealed that diabetes mellitus induced apoptosis of neurons of the basolateral nucleus in the offspring of diabetic mothers that was represented by dark brown coloration of many neurons. A previous study supported our research[42]. A previous research explained that molecular processes could eventually lead to activation of caspase pathway[43].

GABA immunohistochemical staining of developmental ages revealed decreased GABA expression in the 10 and 21 day aged rats of the diabetic lactating mothers. Importance of this finding came from that decreased GABA was found to be associated with autism spectrum disorder which is an important disease of our era. Decreased GABA in amygdala was reported in autistic animals and autistic patients thus GABA could be a therapeutic target in autism[44,45,46]. Recent study also reported that decreased amygdala GABA neurotransmission in autism could explain the associated epilepsy in cases of autism[47].

Previous researches stated increased liability for developing autism in offspring of diabetic rats. Decreased GABA in our study in the offspring of diabetic mothers could be an indicator of increased risk of autism in these offspring[44,45].

As regard the molecular studies, the 21 day old rat amygdala of diabetic mother revealed decreased PPAR gamma expression and this came in accordance with a study that reported the importance of PPAR gamma in treatment of insulin resistance. Moreover PPAR gamma agonists could be used as a therapy for insulin resistance[9].

During pregnancy, PPARs were found to share in many metabolic activities, thus disturbances in PPARs may be involved as a mechanism of gestational diabetes. Moreover, PPAR agonists and modulators may have potent therapeutic role in cases of gestational diabetes mellitus[48].

Moreover a previous study reported that decreased PPAR gamma could be an associated mechanism in autism[49]. PPAR gamma agonists thus could be suggested as a potential treatment for autistic individuals[50].

As decreased PPAR gamma gene was linked to autism. Clinically, the mean age of autism diagnosis is 60 months according to a systematic review and meta analysis that was performed during the period from 2012 to 2019. Usually autism diagnosed later at older ages or sometimes diagnosed at adolescence. So the study of the gene was performed on the 21 days old rats (adolescent rats) to get well established and confirmed results[51].

PPAR gamma agonists act through modifying transmission in synapses in amygdala through alteration of genes[9]. PPAR gamma activation leads to increase in enzymes that lead to increased mitochondrial fatty acid oxidation[20]. It is important to take consideration of this mechanism as there is an association between fatty acid oxidation defects and neurodevelopmental diseases including autism spectrum disorders[52].

The present work revealed that diabetes mellitus caused detrimental effects on the basolateral nucleus in the amygdala of the offspring of diabetic mothers.

Dysfunction of certain nuclear factors by gestational diabetes was reported to cause diverting the developmental events from neurogenesis to gliogenesis[14].

In this work, resveratrol was used to study its possible protective role on the brains of the offspring of diabetic mothers. Resveratrol had the capability of crossing placental barrier with resultant neuroprotective outcomes on the brains of the offspring of the diabetic mother[17]. Resveratrol is also excreted in milk[18], and it also can cross the blood brain barrier[19].

In group IV, histological examination of neurons within the basolateral nucleus showed apparent improvement of principle neurons and stellate cells with few neurons showed little degenerative changes. A previous study reported that resveratrol protect against diabetic embryopathy[53]. Moreover ultrastructural study of this group demonstrated that the pyramidal neurons approximated those of the control rats. Previous studies reported that resveratrol had the capacity to prevent neural tube defects and to restrict teratogenic effect of gestational diabetes on the offspring[54].

The histological results of group IV were confirmed by the morphometric measurements. Resveratrol was found slightly increase the number of neurons in comparison to diabetic rats. It was reported that resveratrol treatment prevented oxidative stress and apoptotic processes that were related to diabetes[55].

Resveratrol was found to decrease caspase-3 immunohistochemical staining of the amygdala. Previous researches reported that the offspring of diabetic rats treated with resveratrol witnessed decreased caspase-3 activity and decreased apoptosis[17,55].

In the current work, it was found an increase in GABA expression in resveratrol treated diabetic group. Resveratrol could be used as protective agent against autism through increasing GABA[56].

In addition, the molecular study revealed increased PPAR gamma concentration in the resveratrol treated diabetic group. Resveratrol was reported as a natural PPAR gamma agonist. PPAR gamma and GABA were decreased in the diabetic group, while they showed an increase in resveratrol treated diabetic group. Thus there is a link between PPAR gamma and GABA[9]. Some investigators suggested the resveratrol as a therapeutic agent in autism spectrum disorders[20].

Several mechanisms explained the neuroprotective effect of resveratrol on the offspring of diabetic mothers. Resveratrol ameliorated the effect of gestational diabetes by activating 5′-AMP-activated protein kinase that is followed by diminished glucose-6-phosphatase in offspring[57].

It was reported that with resveratrol use, pancreatic beta cell showed increased number with increased insulin secretion. Resveratrol also decreased disturbances in protein and lipid metabolism in diabetic rats[58]. Resveratrol was also reported to enhance antioxidant enzymatic activity in diabetic animals thus resveratrol has antioxidant activity[59].

Previous studies also stated that resveratrol treatment decreased inflammation in pancreatic cells and embryonic cells in the offspring of diabetic mice[17]. Anti inflammatory role of resveratrol came through the inhibition of pathways involved in apoptosis cascade through increasing cytokines gene expression[58].

CONCLUSION

Diabetes of the mother during pregnancy and lactation induced severe degenerative changes in basolateral amygdala nucleus of their offspring. These changes could increase the risk of the autism in the children.

Resveratrol administration ameliorates these degenerative changes. Therefore, it can be suggested as a protection for children from autism spectrum disorder in diabetic mothers during pregnancy and breastfeeding.

CONFLICT OF INTERESTS

There are no conflicts of interest

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الملخص العربى

تأثير داء السكري المستحث علي تطور وبنية النواه القاعدية الوحشية في الجسم اللوزي في ذرية الجرذان البيضاء والدور الوقائي المحتمل للريسفيراترول

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مقدمة: يعتبر داء السكري مشكلة مرضية عالمية. يؤثر داء السكري علي العديد من انسجة الجسم وخاصة المخ. ويؤثر داء السكري في الامهات علي المواليد كما يؤدي الي عيوب خلقية.
الهدف من الدراسة: دراسة تأثير داء السكري في الأمهات علي تطور وبنية النواه القاعدية الوحشية في الجسم اللوزي في الجرذان المولودة. كذلك تم دراسة الدور الوقائي المحتمل للريسفيراترول.
مواد وطرق البحث: تم عمل دراسة تنموية حيث تم استخدام ٤٠ من اناث الجرذان. وتم تقسيمها الي اربع مجموعات فرعية علي النحو الآتي:
١-مجموعة ضابطة: شملت ١٠ اناث من الجرذان التي لم تتلق اي علاج.٢-مجموعة داء السكري: تم حث ١٠ من الإناث لداء السكري وذلك بحقن مادة الوكسان بجرعة واحدة (١٥٠ مجم / كجم) داخل الصفاق.٣-مجموعة معالجة بالريسفيراترول: تم اعطائها الريسفيراترول بجرعة ٢٠ مجم/كجم بالفم يوميا بداية من اليوم الاول للحمل واستمر اعطاؤه طول فترة الحمل والرضاعة (حتي اليوم ٢١ بعد الولادة).٤-مجموعة داء السكري المعالجة بالريسفيراترول: تم حث داء السكري في اناث الجرذان مع اعطائها الريسفيراترول بجرعة ٢٠ مجم/كجم بالفم يوميا بداية من اليوم الاول للحمل واستمر اعطاؤه طول فترة الحمل والرضاعة (حتي اليوم ٢١ بعد الولادة).
وفي نهاية التجربة تم التضحية بالجرذان المولودة عند الاعمار الآتية: عمر يوم وعمر ١٠ ايام وعمر ٢١ يوم وقد تم اجراء التقنيات الآتية للنواه القاعدية الوحشية في الجسم اللوزي: الميكروسكوب الضوئي والميكروسكوب الالكتروني. كذلك الدراسات المناعية : باستخدام الكاسباس ٣ وGABA ودراسات جزيئية (جينية): تم دراسة التعبير الجيني ل (PPARγ). ايضا تم عمل دراسات مرفومترية
النتائج: اظهرت المجموعات الضابطة ان تنظيم الانوية الرئيسية تم التعرف عليه في كل الاعمار وان الخلايا كانت صغيرة الحجم ومتزاحمة في الجرذان حديثة الولادة وفي الاعمار الاكبر اصبحت الخلايا اكثر تطورا. المجموعات التي تم فيها حث داء السكري اظهرت الخلايا وجود تغيرات تنكسية, كذلك اظهرت الدراسات المناعية زيادة في انتاج الخلايا للكاسباس ٣ وقلة في تركيز GABA , كما اظهرت الدراسات الجزيئية قلة كمية الجين المدروس ((PPARγ و اظهرت الدراسات المرفومترية انخفاض بليغ في عدد الخلايا مقارنة بالمجموعة الضابطة. في المجموعات التي تم اعطائها الريسفيراترول اظهرت دراسات الميكروسكوب الضوئي والدراسات المرفومترية نتائج مماثلة للمجموعات الضابطة. في المجموعات التي تم فيها حث داء السكري مع اعطاء مادة الريسفيراترول أظهرت الدراسات تحسنا واضحا مقارنة بمجموعات داء السكري.
الخلاصة: يؤثر داء السكري في الامهات بالسلب علي النواه القاعدية الوحشية للجسم اللوزي في ذريتها .كما وجد أن داء السكري في الأمهات يؤدي الي حدوث التوحد في الأطفال المولودة. ويعتبر الريسفيراترول مضاد للاكسدة الذي يقي الجسم اللوزي من التأثير السلبي لداء السكري. كما يمكن إدخال الريسفيراترول في علاج التوحد.