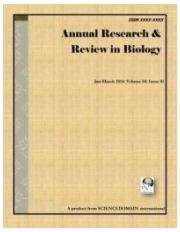

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Embryonic Central Nervous System Development under Morphine Influence in Pregnant Rats as the Epigenetic Factor

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Authors' contributions

This work was carried out in collaboration between all authors. All authors read and approved the final manuscript.

Review Article

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ABSTRACT

Excessive use of narcotic drugs, especially its effect on the central nervous system (CNS) development of the embryos, has given rise to numerous studies in this field. According to the previous studies, morphine consumption during pregnancy can cause a delay in normal development of placenta and embryo. Based on the studies conducted on pregnant mothers' embryo most abnormalities caused by morphine consumption are related to normal development defects of the embryo's (CNS). Drug consumption is one of the factors that involve in epigenetic modifications. Epigenetic bioenvironmental effects such as incompetent nutrition, maladies, infections, stressors and drugs consumption, are some of instances that affect safety and genesis of embryo's (CNS). Survey of epigenetic changes role in normal genesis of embryo's (CNS), is inclusive of world new researches. Identity of different factors of epigenetic modifications is effective in embryo's (CNS) health. Changes induced by epigenetic factors can be moderated or reversed by controlling the epigenetic factors. Based on studies, major epigenetic modifications are

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relevant to DNA methylation and histone modifications. Most important way in prevention from maladies and malformations that result from epigenetic modifications is recognition of epigenetic factors. This paper is a review of the studies on morphine's role in the abnormal development of different parts of an embryo's nervous system .These abnormalities are mainly visible in opioid-dependent mothers and fetuses and include brain nuclei, brain ventricles, choroid plexus, cerebellum, spinal cord, and vision, olfaction , taste deficiencies and epigenetic.

Keywords: Embryo CNS development; brain nucleus; senses; morphine; epigenetic.

ABBREVIATIONS

CNS: Central Nervous System; BBB: Blood-Brain Barrier; CSF: Cerebro Spinal Fluid; CP: Choroid Plexus.

1. INTRODUCTION

Opioids have been used as painkillers for thousands of years. Ancient Egyptian papyrus records reported the use of opium for pain relief [1-2]. In 1973, Candace Pert, used radioactive morphine to evaluate the location of the site of morphine action, and found, surprisingly, that the drug attached to very specific areas of the brain, dubbed "morphine receptors. The primary activity of morphine occurs in morphine receptor or mu receptors. Opioid receptors are activated within the central nervous system (CNS) as well as all over the peripheral tissues. These receptors are normally produced in response to toxic stimulation [1,3-5]. Alkalized morphine crosses the blood-brain barrier (BBB) and increases the fraction of non-ionized morphine. It is notable that brain concentrations of morphine will be enhanced following respiratory acidosis in which cerebral blood flow will be increased to higher carbon dioxide tension and release of the non-ionized form to BBB will be facilitated [5-7]. The widespread abuse of narcotic drugs and their side effects, especially on the nervous system of fetus of pregnant women, has led to numerous researches in this field. Previous studies showed that morphine consumption during pregnancy can delay the normal development of placenta [8-11]. According to research on the fetus of pregnant women, most of the opioid-related disorders are caused by defects in normal development of fetus central nervous system (CNS) [7,9,12-13]. Dependence upon or addiction to these drugs and of special importance, anomalies induced by these drugs in fetus has lead researchers to carry out their researches in this field [14-16]. Today, with the increasing rate of drug addiction in the world, these drugs have become one of the main threats for human health [15,17-18]. Pregnant opioid abusers may unwontedly deliver opioid to their fetus, hence, it is likely that the side effects of opioid abuse during pregnancy, are not limited to the abuser herself and may also affect the next generation. Morphine can easily cross the placental barrier and induce its effects on the fetus. In addition, the drug crosses the blood-brain barrier (BBB) which can lead to several abnormalities in fetus CNS [6,19-23]. Epigenetic modifications can be caused by various environmental elements such as social-psychic stresses, diet, infections, drugs, structural heredity, and gene location in chromosomal width. Research into the causes of epigenetic modifications is very important because epigenetic affects the quality of life and can transmit to the next generation via inheritance [24-25] .Epigenetic factors including drug consumption, unsuitable nutrition, and horrible bioenvironmental situations, can cause epigenetic modifications in embryos CNS. Principal epigenetic modifications are relevant to external factors [26-28]. In mammals, DNA

methylation and histone variations are the most prevalent epigenetic modifications [28-29]. The main objective of this review is to survey the fetal nervous system defects resulted from morphine consumption by pregnant female rats. The studies conducted have examined the role of morphine on development of different parts of fetal CNS including the cranial nuclei, cerebral ventricles, choroid plexus, cerebellum, spinal cord, vision, olfaction and taste and also analyzed the morphological changes of neural tissues on special days of pregnancy with microscopic observations [30-36].

Figures: Fig. 1 (C_1, M_1) and ($C_{1\text{-}1}$,$M_{1\text{-}1}$), Fig. 2 (C_2,M_2), Fig. 3 (C_3,M_3) and ($C_{3\text{-}1}$,$M_{3\text{-}1}$), Fig. 4(C_4,M_4) , Fig. 5 (C_5,M_5) and ($C_{5\text{-}1}$,$M_{5\text{-}1}$) and ($C_{5\text{-}2}$,$M_{5\text{-}2}$), Fig. 6(C_6,M_6), Fig. 7 (C_7, M_7) and ($C_{7\text{-}1}$,$M_{7\text{-}1}$) , Fig. 8 (C_8, M_{87})

1.1 Development of Neural Plate and Neural Tube under Morphine Influence

Precordial notochord and mesoderm induce the overlaying ectoderm to thicken and to form neural plate. The nervous system is more susceptible to damage caused by consuming drugs such as opioids in the early stages of embryonic development, which have the greatest impact on development of the neural ectodermic cells [37-38]. Previous research on oral morphine consumption by pregnant mothers showed that it delays the development of embryonic layers (ectoderm, mesoderm, and endoderm). Following a defect in ectoderm layer, formation of neural plate and neural tube in the ninth day of pregnancy impairs [38-39]. The existing literature demonstrates that morphine consumption during the ninth day of pregnancy prevents neural fold from transforming in to neural tube by blocking the epithelial receptors in CNS. Subsequent investigations revealed that mothers' addiction to morphine, particularly during early pregnancy, reduced birth weight, produced neural tube defect, and possibly caused neurological abnormalities including exencephaly, cranioschisis, brachyury, and spinabifida [40-46].

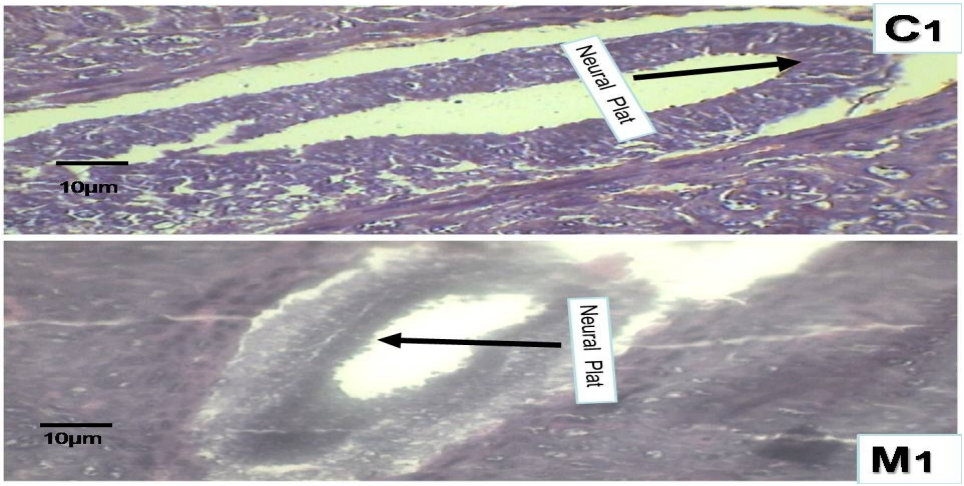

Fig. 1(C,M). Delay in neural plate development in morphine treated in comparison with untreated groups in pregnancy 9th- day [38]. Experimental (M_1) and control (C_1) groups

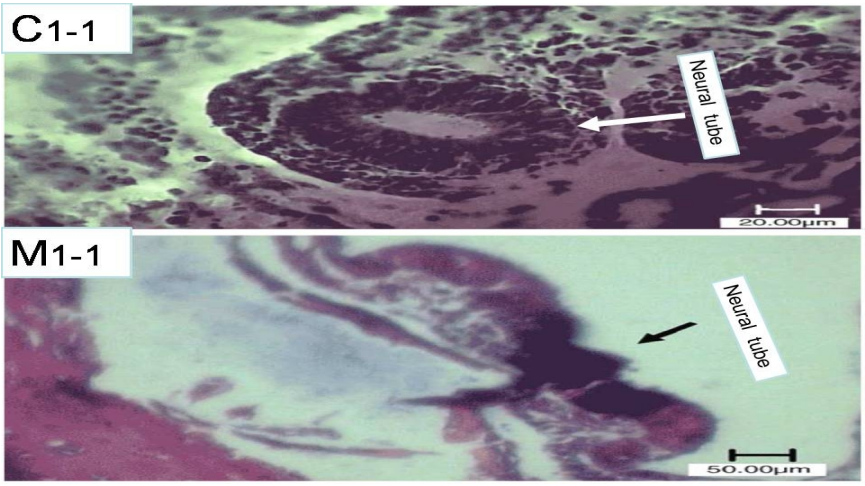

Fig. 1.1(C,M). Delay in neural tube development in morphine treated in comparison with untreated group in pregnancy 9th- day [40]. Experimental (M_{1-1}) and control (C_{1-1}) groups

1.2 Development of Spinal Cord and Ependymal Duct under Morphine Influence

The nervous system develops initially as a neural tube with a narrow part called spinal cord and a wide cephalic part called brain vesicles. Spinal cord acts as a mediator between muscles and organizing center of the body and has a major role in transmission of motor and sensory messages [45-46]. Based on the previous studies, morphine consumption by pregnant mothers disrupts the normal development of spinal cord in both parts of white and gray matter [12,17]. The ependymal canal is surrounded by primary nerve cells or neuroblasts which have high a division potency [47-48]. Accumulation of neuroblasts around ependymal canal leads to the formation of gray matter of the spinal cord. The white matter of spinal cord includes the outermost layer of the spinal cord which contains neuroblast nerve fibers. Oral morphine consumption by pregnant mothers increases abnormal cells in mental part of embryo brain [49-50]. On the other hand, any disruption in the development and function of the spinal cord leads to disturbance in perception of sensory messages from organs and in transmission of motor messages to muscles [48,51-52]. This can cause major sensory and motor abnormalities in infants born from morphine-dependent mothers. Research has shown that morphine reduces the ependymal canal surface in fetal brain of mothers addicted to morphine [46,49]. Considering that cerebral cavities and canals are responsible for transferring cerebrospinal fluid(CSF) and for feeding neurons, any defect in ependymal canal development leads to defects innerve cells development [53-54]. Any abnormality in the development of mental and marginal parts impairs sensory and motor neurons. Thereby, morphine consumption severely reduces the surface of primary brain vesicles (prosencephalon and rhombencephalon) and increases the thickness of brain cortex [49,55].

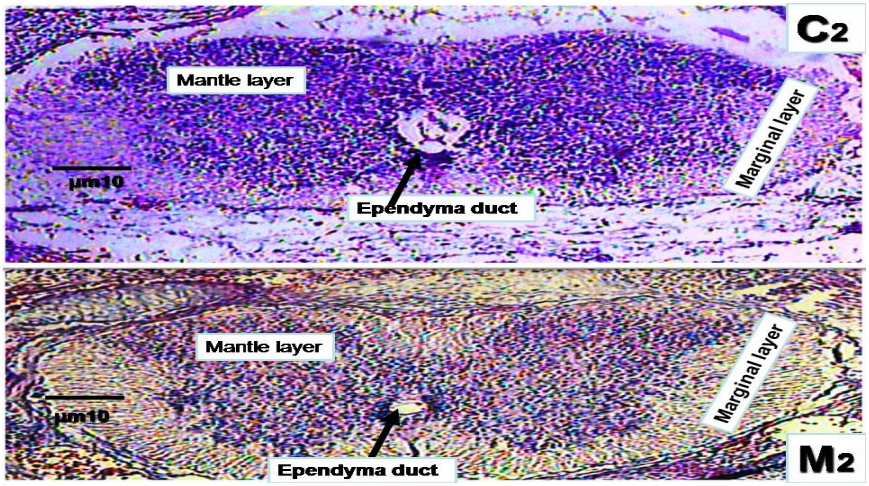

Fig. 2(C,M). The development of spinal cord and ependymal duct. Reduced the ependymal canal and gray matter surface in pregnancy 17th- day morphine-treated groups [31]. Experimental (M_2) and control (C_2) group

1.3 Development of Brain Vesicles and Ventricles under Morphine Influence

Normal development of the neural tube continues by formation of three swells at the cephalic end of the neural tube including prosencephalon, mesencephalon, and rhombencephalon, called primary brain vesicles [46,49-50]. Research has shown that the development of neural tube in rat embryo is accomplished on the ninth day and that of primary brain vesicles on the tenth day of pregnancy. Since the central canal and cavities are the transferring pathways of nutrients (CSF) of the nervous system, they play a major role in the development and functioning of neural cells [48-49]. Studies about the role of oral morphine consumption in the development of cerebral ventricles of the fetus of pregnant rats indicated that morphine reduces the surface of lateral ventricles and the third ventricle in 17 day -old embryos. Further investigations revealed that oral morphine decreases the surface of the fourth ventricle in 14 day-old embryos and increases the thickness of brain cortex [31,53-54]. Cerebrospinal fluid flows from lateral ventricles into the third and then the fourth ventricle and ultimately enters the central canal. Thus, any factor disturbing the normal transmission of CSF within the cavities and central canal is considered as an interferer in the development of nerve [56-57]. Since the central duct and ventricle transfer nutrient materials to the nervous system and cerebrospinal fluid, any disorder in cerebrospinal fluid pathway from lateral to third ventricles due to Monro hole blocking would cause an increment in the third ventricle and the surface of cerebral cortex would shrink [53-54,56]. Decrease in ventricles and the central canal surfaces can lead to reduction of the cerebrospinal fluid formation that may result in irreparable anomalies in the nervous system functioning [56,58-59]. Symptoms of oral morphine consumption by pregnant women are in conflict with congenital hydrocephalus in which surface of the brain cavities and central canal are decreased due to morphine administration [31,56]. Abnormal cerebrospinal fluid decrease results in a reduction in ventricles and central canal surface. Morphine as an interferer in the normal development of the choroid plexus ependymal cells abnormally increases the number of cells [46].

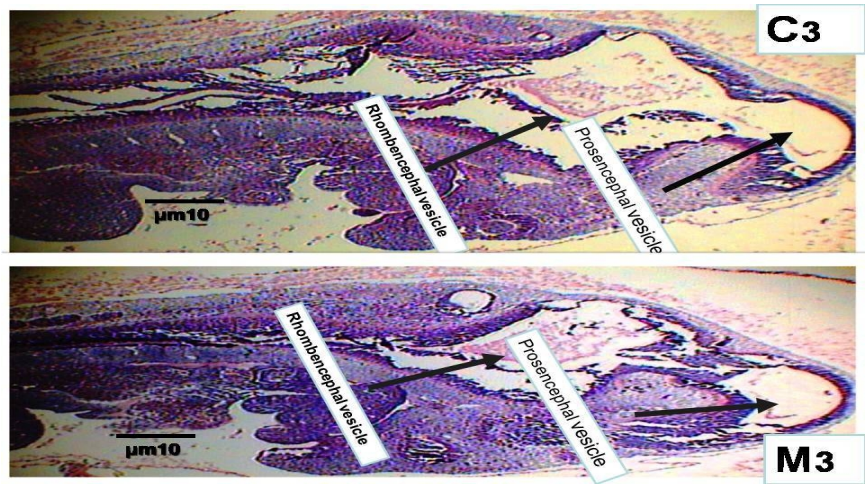

Fig. 3(C,M). Brain Vesicles development. The surface reduction of primary brain vesicles (Prosencephalon, Rhombencephalon) in pregnancy 10th- day morphine-treated group [32].Experimental (M_3) and control (C_3) group

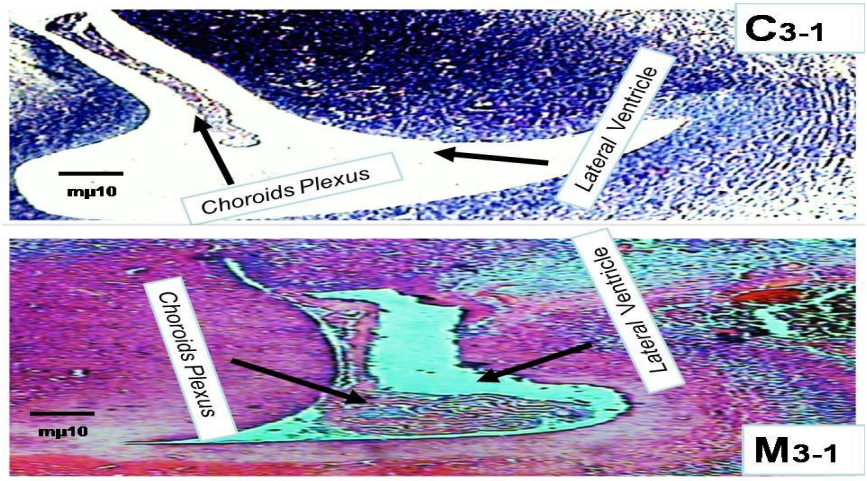

Fig. 3.1(C, M). Development of brain ventricles and choroid plexus in rat embryo. Decrement in brain ventricle surface and increment in choroid plexus surface are clearly visible in morphine treated group compared with untreated group in pregnancy 17th- day [31]. Experimental (M_{3-1}) and control (C_{3-1}) groups

1.4 Development of Ependymal Cells and Choroid Plexus under Morphine Influence

Myelencephalon roof plate contains a single layer of ependymal cells which has evolved to pia mater and ultimately leads to the creation of Choroid plexus (CP). CP which feeds the nerve cells is formed by ependyma cells covered by mesenchymal vascular. To accomplish the task of generating sufficient CSF, choroid plexus tissue receives a large blood supply relative to its size. In addition to CSF production, the CP acts as a filtration system, removing

metabolic waste, external substances, and the excess of neurotransmitters from the CSF [60-62]. Cerebrospinal fluid is synthesized, absorbed, and secreted in the nervous system by choroid plexus. Based on previous studies, oral consumption of morphine by pregnant rats can delay the normal development of fetal choroid plexus. Morphine as an interferer in the normal development of the choroid plexus ependymal cells abnormally increases the number of cells [31,62-63].Studies using radioactive morphine have shown that morphine effect is most of all concentrated in the endothelial membrane of blood cells, where opioid receptors (μ, κ, and δ) are located. Therefore, destructive effects of morphine function are high in hyperemic parts. Since the function of choroid plexus is crucial for the development and supply of brain cells, its morphine-induced abnormalities play a major role in developmental defects of the nervous system cells [4,9,32,64].

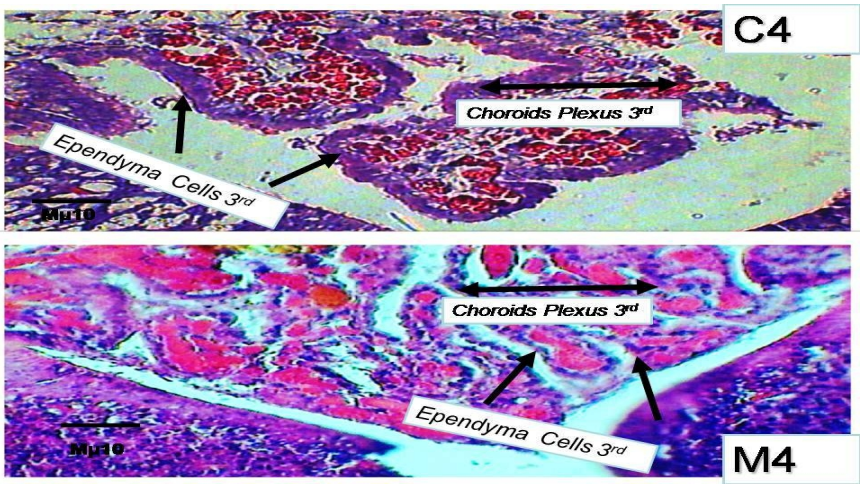

Fig. 4(C,M). Ependymal cells in rat embryo. The number of ependymal cells and intracellular space has been increased inpregnancy 17th- day morphine-treated groups [62]. Experimental (M_4) and control (C_4) group

1.5 Development of Hippocampus, Amygdale and Basal Ganglia under Morphine Influence

Hippocampus is an important part of the limbic system which plays a crucial role inattention and alertness. Defects in the development of hippocampus can lead to learning and memory dysfunction. Several studies have reported that neonatal morphine administration would alter the neurochemical profile of the developing hippocampus and decrease neurogenesis in the dentate gyrus [40,65-66].According to the research, oral morphine consumption during pregnancy can delay the normal development of hippocampus layers. Moreover, morphine increases the number of hippocampus cells and also plasma corticosterone levels, both of which can trigger abnormal cell division. These changes may explain the enduring neurobehavioral deficits seen in adulthood followed by neonatal morphine administration [67-69]. The amygdaloid complex is a brain structure that plays a major role in modulation of neuro-hormonal activity, visceral reflexes, and complex behavioral patterns like nutrition, defense, nervousness, reproduction, memory, and learning [70-71]. Oral consumption of morphine by pregnant rats can impair the normal development of fetal amygdala complex, for instance decreased size and increased number of the amygdaloid cells. Impairment of amygdala cell development will also affect neuron function resulting in changes in animal

behaviors such as feeding, reproduction, and fear after birth [13,70,72].The development of basal ganglia nucleus is accomplished in diencephalon. Non-instinctive movements (dorsal basal ganglia) and instinctive movements (ventral basal ganglia) are regulated by basal ganglia [73-74]. Morphine can impair the normal development of fetal basal ganglia. Considering that the development of basal ganglia begins on the 12th day of pregnancy, previous studies have shown that morphine not only delays its development process but also increases the thickness of all the three layers. Impairment of ganglia development results in motor abnormalities in infants born by addicted mothers [73,75].

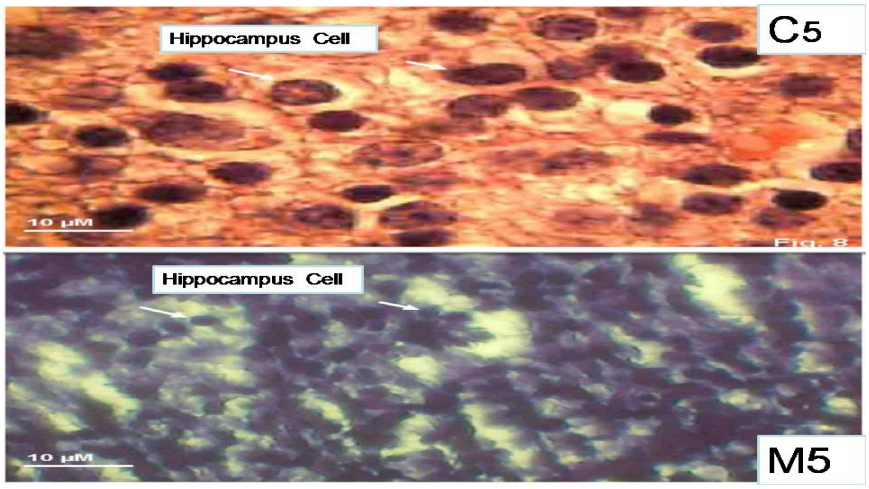

Fig. 5(C,M). Hippocampus development. The number of hippocampus cells have been increased and intracellular space has been increased in morphine-treated groups in pregnancy 19th- day [30]. Experimental (M_5) and control (C_5) groups

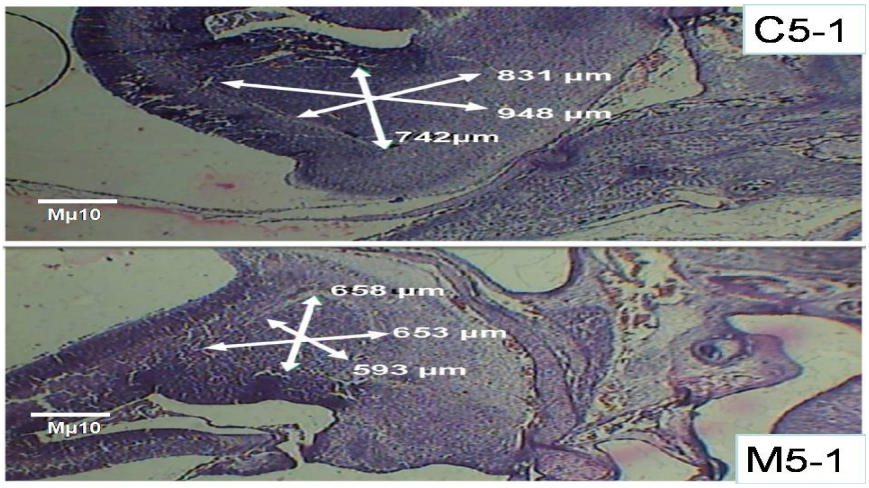

Fig. 5.1(C,M). Amygdalacomplex development. Decreased area and all size of the amygdala complex in pregnancy 19th- day of morphine-treated groups [70]. Experimental (M_{5-1}) and control (C_{5-1}) group

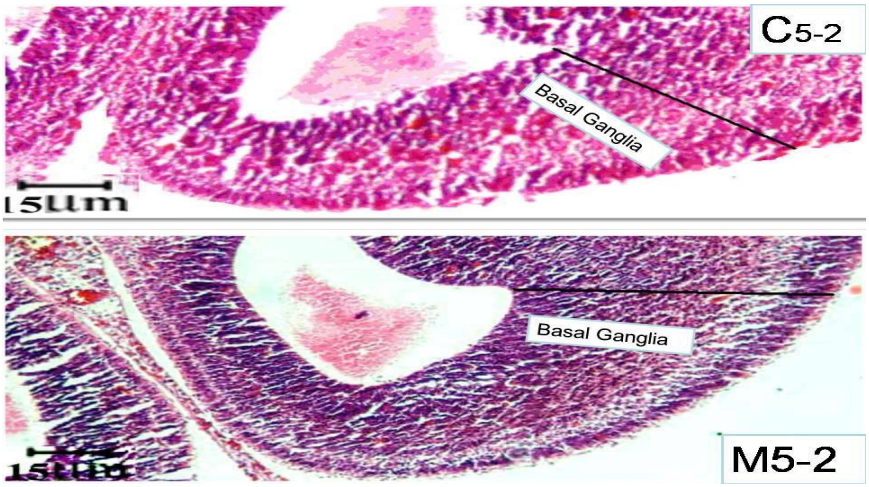

Fig. 5.2(C,M). Basal ganglia nucleus development. Morphine impairs the normal development of the Basal ganglia compared with untreated groups in pregnancy 17th-day [73]. Experimental (M_{5-2}) and control (C_{5-2}) group

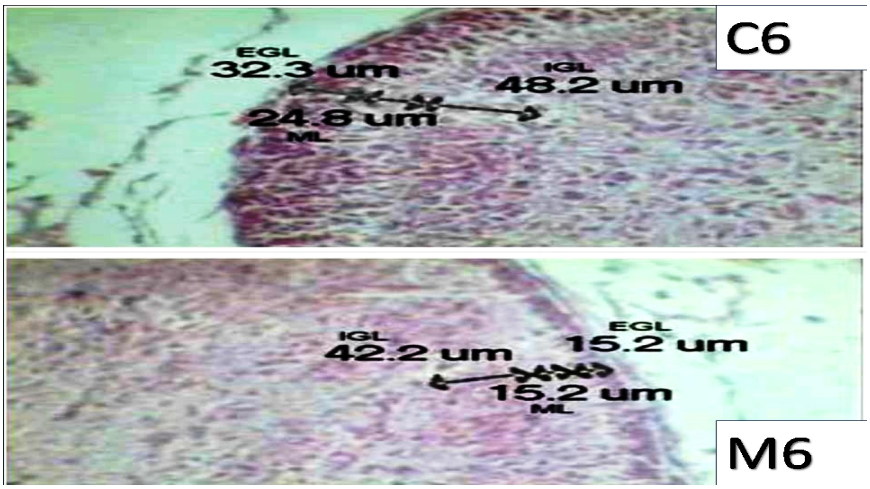

Fig. 6(C,M). Cerebellum development. The thickness ofCerebellar cortical layers, especially that of the external granular layer (EGL) and. Internal Granular Layer (IGL), was decreased in pregnancy 17th- day of the morphine-exposed embryos [76]. Experimental (M_6) and control (C_6) groups

1.6 Morphine Delays Cerebellar Development under Morphine Influence

Being the center of balance and regulation of body movements, cerebellum is rooted in metencephalon area of the brain. In the case of rats, the original cerebellum neurons are proliferated at the end of the third week of pregnancy, just like granular cells. Studies show that morphine consumption disrupts the normal development of the cerebellum in the embryo [5,76]. Morphometric and cell counting findings of this study showed that morphine consumption during pregnancy inhibits cell proliferation in outer granular layer of the

cerebellum and slows down neuronal migration in the inner layer towards the cortical area. Moreover, morphine administration on pregnant rats during pregnancy could reduce cerebral cortical growth of the embryo [16,76-77]. It seems that opioids do not allow the cells to proliferate adequately at the stage when they are strongly replicating. Therefore, morphine administration during pregnancy may disrupt the replication and neuronal migration in the cerebellum cortex of the embryo [33,77]

1.7 Development of Olfaction and Taste under Morphine Influence

The olfactory system differentiation depends on the epithelial-mesenchymal interaction. These interactions take place between the cells of the neural crest and ectoderm of fronto-nasal swell to form the olfactory fossa. Olfactory bulb is also formed between crest cells and telencephalon floor. The olfactory tract is located behind the ethmoid skull bone [69,78]. Olfactory nerve fibers pass through this bone and enter the olfactory bulb and end at glomerulus surface. The layer contains mitral and tufted cells the axons of which form the cranial nerve in mice. The nerve ends at primary olfactory cortex and other areas of the brain. Formation of different cells of olfactory bulb shows a precise and regular temporal pattern. The first neurons found in this area are mitral cells that are formed on 14 th to 16th days of fetal. Olfactory bulb as the concentration center of olfactory information is of great importance to the animal. Studies show that oral administration of morphine in pregnant rats reduces the antero-posterior length and the weight of embryos. Microscopic observations show that morphine impairs the normal development of the olfactory bulb [69,79]. Three different cells are detected in olfactory bulb layers called spherical, dark, and pyramidal cells; but their types remain un known since their developmental stages are not yet completed. During the development of olfactory bulb, morphine induces cell transformation, reduces the number of cells, and increases the intercellular space. Morphometric observations have shown that the thickness of the outer and middle layers increases and that of the inner layer decreases. Defects in the development of olfactory bulb impair the transmission of olfactory messages to olfactory cortex [12,59,79]. The olfactory cortex participates in the most important functions of the brain for survival through circuits that are created after birth. This area of the brain plays a significant role in creating and sustaining cognition and cognitive behaviors. Studies have shown that morphine delays the development of three layers of neuronal cells of the olfactory cortex and reduces the density of neurons and neurons projections [78-79].Together, these results demonstrated that acute olfactory impairment could attenuate already established addiction-related behaviors and expression of c-Fos in drug-addiction-related brain regions, perhaps by affecting the coordination between reward and motivational systems in the brain [69,79-80]. Taste communicates with other sensory systems, especially with the sense of smell, as well as trigeminal nerve and plays an important role in survival of organism. Meanwhile, the tongue plays a particular role in the proper functioning of this sense due to its taste receptors [81-82]. Exposure of the fetus to abnormal foreign material during tongue development may delay or impair its development resulting in abnormal functioning of the gustatory system. Mother's dependence on morphine reduces the large diameter of the tongue, increases the number of cells, and reduces cells size [36,83].

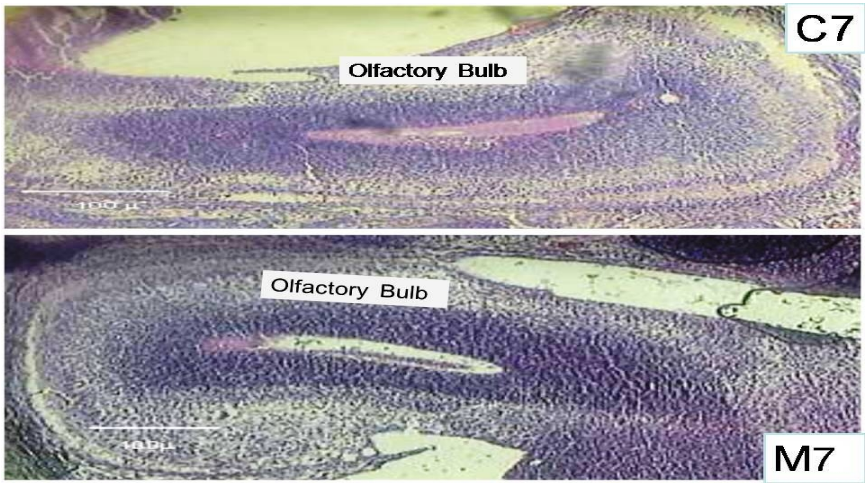

Fig. 7(C,M). Olfactory bulb development. Morphine impairs the normal development of the olfactory bulb compared with untreated groups in pregnancy 19th- day [79].Experimental (M_7) and control (C_7) group

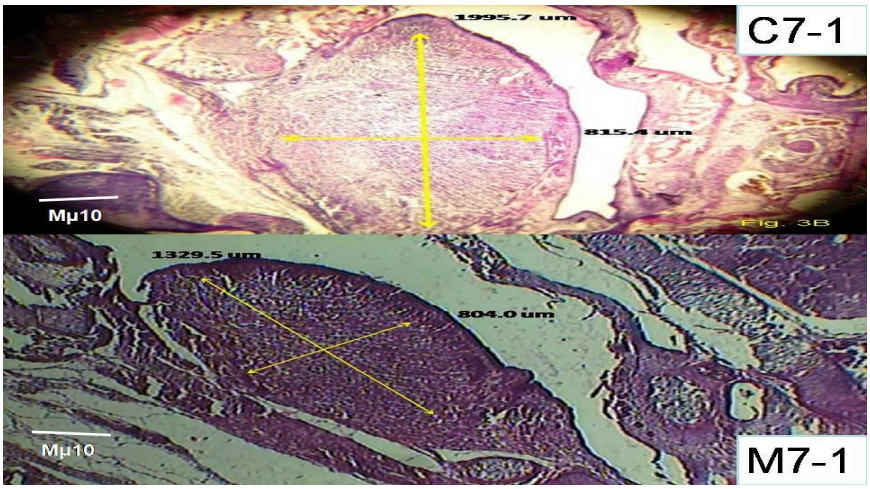

Fig. 7.1(C,M). Tongue development. The large diameter of the tongue was reduced in morphine treated group in pregnancy 19th- day [36].Experimental ($M_{7\text{-}1}$) and control ($C_{7\text{-}1}$) group

1.8 Development of Sight Sense of Rat Embryo under Morphine Influence

Development and formation of visual system begins with diencephalon of forebrain as optic vesicles. Retina is the neural part of the eye and its proper development plays a major role in visual system function. Retinal thickness reduction, malformation of retinal layers, and delay in retinal cell differentiation are the effects of oral morphine consumption by pregnant women on visual system function [35,49,84]. Moreover, the overall size of fovea region and its central thickness that is connected to the optic nerve is reduced. In addition, morphine administration during pregnancy impairs fetal normal development of the lens which is visible

as a decrease in the lens length and an increase in the eyelid thickness [85-87]. Morphine also increases migration of lens cells from around toward the center. Regarding morphine-induced increase of plasma corticosterone, the anomalies of visual system development resulted from morphine administration can be attributed to corticosterone intervention. Corticosterone has a very broad impact on development, proliferation, and migration of cells. Corticosterone causes excessive cellular proliferation, impaired development, and delay in cell migration during embryo development [35,88-91].

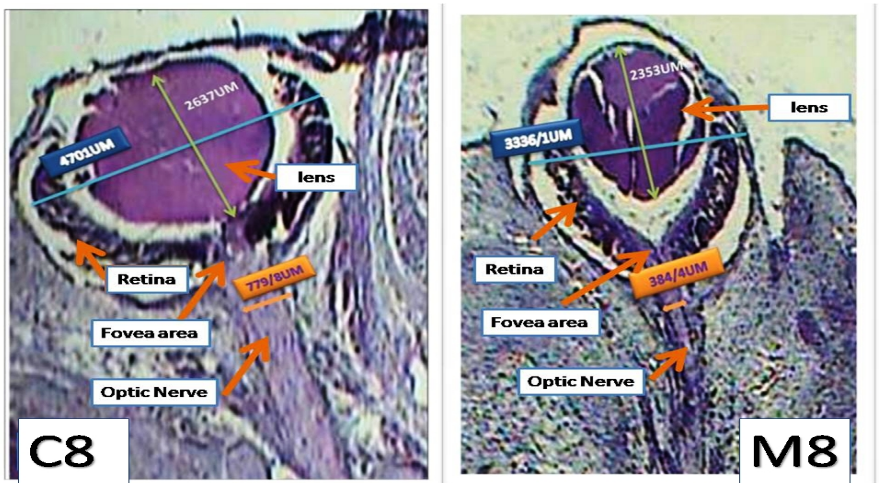

Fig. 8(C,M). Eye development. A decrement in the lens length and an increment in the eyelid thickness were visible in morphine treated groups in pregnancy 17th- day [35]. Experimental (M_8) and control (C_8) group

1.9 Modifications That Result from Morphine Consumption as an Epigenetic Factor on Rat Embryo CNS

Epigenesis is the study of molecular mechanisms that influence how the environment controls the gene's activity. Some factors such as nutrition, drugs, stress, and emotions can change gene expression. Epigenetic factors can turn on or off genes. Morphine is an epigenetic factor that causes epigenetic changes in embryo of mothers that consume opiates [92-94].

Epigenetics is most commonly defined as the study of alterations in gene function that are heritable through both mitosis and meiosis, but do not involve any change in the DNA sequence itself. DNA methylation most commonly occurs at cytosine–guanine dinucleotides (CpG), and is generally associated with transcriptional silencing [93,95-96] Histone methylation on lysine is associated with both actively transcribed and silenced genes depending on the residue. It can occur as mono-, di- or tri-methylation, which modulates gene expression differently. Histone methylation is also found on arginine (R) in both mono- and di-methylated forms, but the impact on chromatin structure is not well understood [25,29].

The role of epigenetic factors in the regulation of placental growth and development. Epigenetic factors such as DNA methylation, histone alternations and non-transcriptional RNAs, are effective in driving gene expression patterns, and these changes have a pivotal

role in the genesis of the embryo CNS [97-98]. Some epigenetic changes such as DNA methylation modifications and histone alterations that occur in the genome, are important mechanisms associated with opiate addiction [95,99]. In addition, molecular and behavioral experiments certify that epigenetic modifications have a role in abuse of different drugs such as cocaine, amphetamine, and alcohol [99-100]. Drugs are one of the important factors that create main epigenetic modifications (DNA methylation and histone alternations). These changes cause disruptions in blood interchanges between mother and embryo as well as with passage from placenta, disrupting embryonic CNS development [99,101-102]. Morphine utilization by pregnant mothers causes a delay in part of embryonic tissue genesis especially CNS tissue of the embryo. Addiction to cocaine and amphetamines leads mostly to acetylation in H3 and H4 histones [99,103-104]. Histone alterations are mostly acetylation and phosphorylation that activates transcription on the lysine, serine, threonine, and tyrosine N-T tails. After extended treatment with morphine, suppression of mu opioid receptor miRNA expression is one of the morphine tolerance mechanisms [99,104].

2. CONCLUSION

Review of several studies depicts the morphine consumption by pregnant rats and its impact on the development of different parts of the fetal nervous system. Following a delay in the normal development of central nervous system, multiple abnormalities arise in its normal function. Epigenesis is the study of molecular mechanisms that influence how the environment controls the gene's activate. Some factors such as nutrition, drugs, stress, and emotions can change gene expression. Epigenetic factors can turn on or off genes. Morphine is an epigenetic factor that causes epigenetic changes in placenta of mothers that consume opiates. The main epigenetic changes are relevant to DNA methylation and, less so, histone alterations. Acquaintance with these factors is an important before treatments. The importance of these studies is that the embryos are very sensitive to intra-uterine period of their lifespan and any extra manipulation during this period can result in an abnormal development of different parts of the central nervous system ;the hazard which must be noted when a considering a new generation.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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