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Histological study of the embryogenesis of metencephalon in pre-implantation of albino rat's embryos after maternal treated with silver nanoparticles

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Abstract: *Cerebellum is the most important and critical part of the central nervous system, cerebellum is very sensitive to the abnormal changes during the embryological development in its histological structure, the exposure to any infection during embryogenesis produce abnormalities in the cerebellum and behavioral of offspring. In this study we tried to study the ontogenesis of the cerebellum in the embryos of the albino rats and detection the effect of the AgNPs on the ontogenesis of the rat cerebellum after exposure of AgNPs during pregnancy. we used 60 female pregnant rats divided in to three group, each contain 20 female, (G1) treated with 2mg/kg /day suspension of silver nanoparticles (Ag NPs) (G2) treated with 20mg/kg/day AgNPs from first day of pregnancy until delivery at 21 days and (G3) was considered the control group received D.W only. We were select the embryonic day (ED 7,12,15,18 and 21). The results showed apoptosis, degenerative and dispersion in glial cell in the internal granular layer of cerebellar cortex and less dense of external granular layer due to AgNPs may pass the blood brain barrier (BBB) to the embryo's brain when female exposure to the AgNPs during pregnancy.*

Key words: *embryogenesis, metencephalon, rats, silver, nanoparticles*

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I. Introduction

Because rapid development of nanotechnology has led to the wide application of nanoparticles (NPs) in various fields such as, catalysis and biotechnology including cosmetics, pharmaceutics and medicines (Rivero *et al.,* 2015). As to antibacterial/antifungal characteristics, silver nanoparticles (AgNPs) have been used in clothes, cosmetics, wound dressing, air- freshener sprays, water disinfectant, sunscreens, hygiene products and food containers, which increases the release of nanoparticles to environment and may cause exposure to human. Nanoparticles can cause toxicity, inflammation and oxidative stress after exposure to AgNPs (Prabhu and Poulose, 2012) and (Ribeiro *et al.,* 2013).

Worldwide rate of central nervous system (CNS) disorders is rising along with the age of population and result chemical agent, making it an increasingly relevant subject. Protecting the CNS is the blood-brain barrier (BBB) which, consequently, hampers the success of current therapies by blocking drugs and other chemical and nanoparticles to access to brain (Gomes *et al*., 2016). BBB is a layer of endothelial cells very tightly bound to each other (by tight junctions, TJ) that block the transport of drugs and many nanoparticles from blood to brain (Bhaskar *et al.,* 2010).

The cerebellum is considered one of the more sensitive part of the CNS in mammals because the diversity in the type of the differentiated cells during embryogenesis and its role in the physical and behavioral in the ontogeny therefore the early exposure of the developing cerebellum lead to many histopathological changes (Qin, *et al*., 2006 and Wang *et al*., 2014).

However, there is a lack of information concerning the impact of NPs on human health, as it was proved that the nanoparticles could be administered to human body by several routes including inhalation, ingestion, dermal penetration, and injection, followed by the distribution of these nanoparticles to various tissues through systemic circulation (Pardridgea, 2016) The nanoparticles are small enough to penetrate very small capillaries throughout the body, NP size is important in CNS penetration, with several studies suggesting a 20–70nm diameter as being optimal for transport, Surface charge can also facilitate NP-mediated BBB disruption and therefore they could offer the most effective approach to target certain tissues such as brain and can affect the physiology of any cell in an animal body (Niewoehner *et al.,* 2014) and They also are at high risk of having brain disease such as Alzheimer's disease. (Shilo *et al*., 2015).

II. Materials and methods

2.1Animal housing

In this study, 60 mature female Sprague-Dawley albino rats (*Rattus norvegicus)* were used. It was purchased from animal house of Iraqi national center for drug control and research. The average ages of females were 1.5-2 months age and their average weights between 100 – 300g.

All animals housed in plastic cages with a metal network cover under climate conditions, with temperature 22±2°C and 12:12 light and dark cycle. Rats were provided with water and food *ad libitum.* Cages were cleaned and sterilized in different time with 70% ethanol. After mating .The gestational day zero was defined as the day when spermatozoa were observed in the smear of the vaginal smears, then females were transferred to separate cages without males and stay until the appropriate days to isolate the embryos (Ypsilantis *et al.,* 2009).

60 mature female were divided in to three groups, (G1) received (2mg/kg/ B.wt AgNPs) (G2) received 20mg/kg/B.wt AgNPs and (G3) as control group received D.W only. The AgNPs solution was given orally by using polyethylene orogastric tubes connected to a hypodermic syringe with a volume of 1-2 ml.

2.2 Preparation Silver nanoparticles (AgNPs)

AgNPs it was purchased as grey black solid powder (purity 99.9%, apparent density: 0.97g /ml, tap density: 2.16 g/ml and CAS NO.: 7440-22- 4) with an average diameter of (40-59.71) nm, it was examination under scanning probe microscope. AgNPs was prepared at a two concentrations, low concentration 2mg/kg of body weight and high concentration 20mg/kg of body weight according to (Charehsaz *et al.,* 2016). The AgNPs stock solution was prepared by weight of AgNPs powder in a certain volume of deionized distilled water. The suspension was exposed to the ultra-sonication technique by ultrasonic water bath for 2-3h in dark and under biological safety.

2.3 Embryos retrieved

The pregnant female albino rats were fully anesthetized by diethyl ether for several minutes. The female were killed to remove the embryos in different gestation days (GD7- 12-15-18 and 21). In treated and control groups, abdominal midline incision was performed, the two uterine horns were exposed, the embryos were extracted from the placental sacs by hysterectomy, and the extra-embryonic membranes were then removed, rinsed in normal saline, then the embryo was examined under the dissecting microscope. The ED7 and ED12 transferred immediately to the Bouin's solution for fixation, embryos at ED15, 18 and 21 the skulls were removed by dissecting tools and cerebellum was isolated from brain carefully by incision along the dorsal aspect. All of the samples were fixed in the Bouin's solution, for 24-48h and were transferred to 70% ethanol until the time of the histological section.

2.4 Histological preparation

The preparation of histological sections depends on the standard methods of Allen & Cameron (2004). All samples were passed through progressive increasing concentrations of ethanol (70%, 80%, 90%, 95%, and 100%) with two hour interval; the samples were clearing by toluene for 1h. Tissues were embedded in a melted paraffin wax then all samples stained with hematoxylin and eosin stain, and then examination under light microscope. [13]

III. Results

Serial sagittal sections of the hindbrain and cerebellum were taken from ED 7 to 21 days. The results of the control group in early development from ED7-ED12 showed simple histogenesis of metencephalon anlage and cerebellum anlage , the radial glial cells are not yet ell differentiated, slight thickness of external granular layer (EGL), cellular features in molecular layer, appearance of early purkinje cells layer and dense regular cell in the internal granular layer (IGL) later development (ED15-ED21) show dense ependymal cells, appearance the mantle layer, the result showed dense EGL, and appearance glial cells in IGL were well development at ED21, molecular layer, Purkinje layer and well differentiated IGL "Fig.1A, B ". While, the histological examination of the rat's embryo metencephalon anlage and cerebellum treated with 2mg/kg/B.wt of AgNPs not appear histological abnormalities in the early development compare with control group. While at later development showed narrow in marginal layer, dispersed and irregular glial cell in cortical plate, less wide of intermediate zone and appearance ventricular and sub ventricular layer, the cerebellum showed well appearance of EGL, less cellular in molecular layer and found glial cell in IGL at ED18-21 showed dispersed in EGL, degenerative in purkinje cell layer and dispersed and depletion of IGL compare of control group "Fig. 2 A, B ".

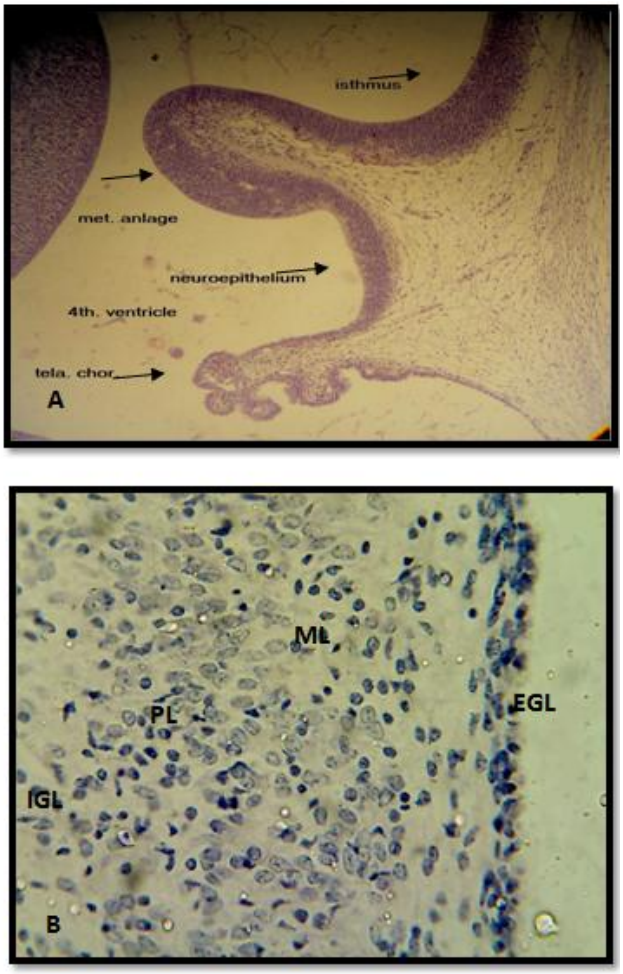

Figure1: sections in rat embryo metencephalon of control group

A- metencephalon anlage at early development (HandE stain 40X)

B- magnified section of metencephalon anlage at early development EGL: external granular layer , ML: molecular layer , PL: purking Layer ,IGL:internal granular layer (H and E stain 400X)

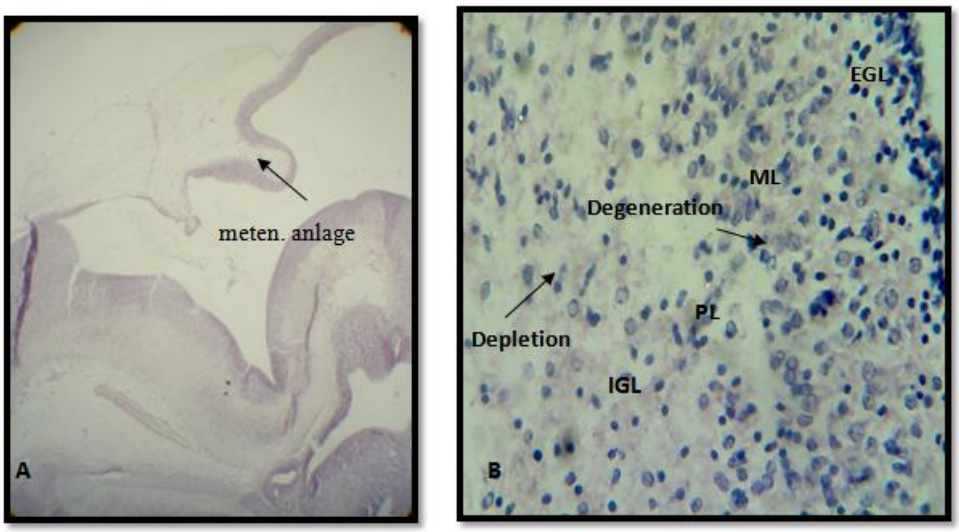

Figure2: section in rat embryo metencephalon anlage and cerebellum treated with 2mg/kg

A- metencephalon anlage at early development (H and E ,40X)

B- cerebellum anlage at late development shows EGL: external granular layer

ML: molecular layer , PL: purking Layer ,IGL:internal granular layer (H and E, 400X)

the histological examination of rat metencephalon treated with 20mg/kg/B.wt AgNPs at early development embryo development showed sever hemorrhage and congested blood vessels in ependymal layer, depletion and degenerative in the proliferation mantle layer cells. Showed less dense of EGL, found of cortical plate, intermediate zone and dispersed sub ventricular and ventricular layer. In the ED15 the results showed apoptosis of IGL, undifferentiated of purkinje layer and narrow, less cellular of molecular layer. The histological section at ED18 revealed marginal layer and dispersed of mantle layer compare of control group. The histological section at ED21 was the uterus only because no obvious embryo we could see when examined the histology of the uterus to detect may absorbed embryos showed not clear lesions in uterus and not showed any implantation at this stage, not obtained any embryos at this stage (Figure 3A, B, C, D and E)

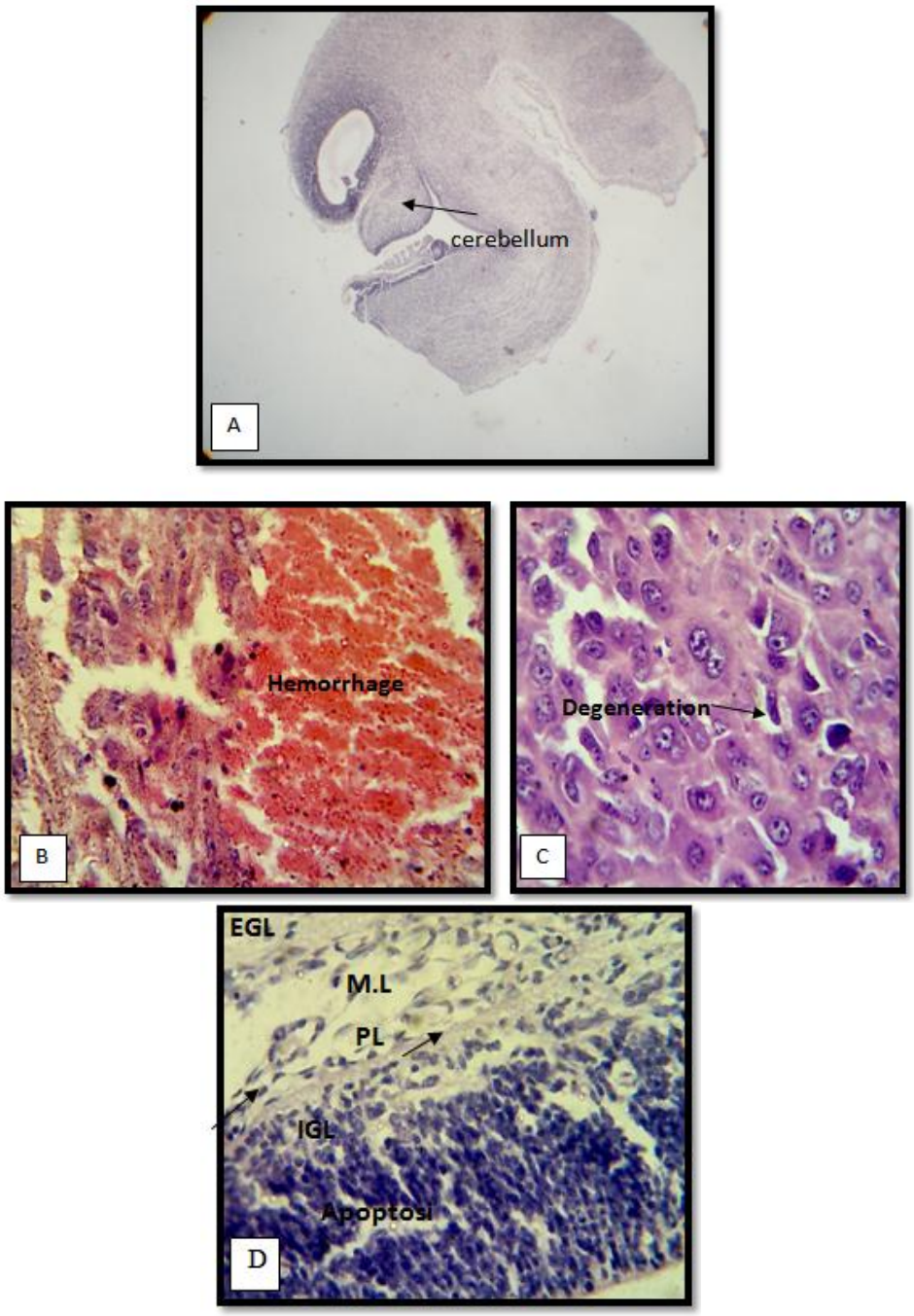

Figure3: section in rat embryo metencephalon and cerebellum treated with 20mg/kg of AgNPs A- metencephalon at early development (H and E stain 40X)
B,C,D - cerebellum analage at late development EGL: extenal granular layer (H&E stain 400X)
ML: molecular layer PL: purkinje layer , IGL:internal granular layer

IV. Disscusion

In the present study, we investigated the effects of different concentrations of AgNPs which average of diameter was (40-59.71nm), on the histological changes of the metencephalon during the embryogenesis.

The AgNPs were showed some affected on gestational parameter including abnormalities and malformation in some organs in embryos and implantation (Charehsaz *et al*., 2016) they found that gestational parameter did not affected by AgNPs treated dose compared hazard of AgNO3 administration.

On the other hand, some researchers showed that the numbers of nonviable fetuses was significantly increased in dams treated to a single oral dose of 10 mg/kg/B.wt AgNPs not higher doses, this due to facilitation of aggregation AgNPs at higher concentration in gut and preventing internalization via the gastrointestinal tract and so reducing fetotoxicity at higher doses (Philbrook *et al*., 2011). our results revealed obviously toxicity of AgNPs of embryos brain because this nanoparticles can be absorbed, and then accumulated in the brain regions by passing through the (BBB) during the development of brain when the barrier is not full formed, this may be cause of some histological changes which occur in the development of early cerebellum of the embryos mainly in the mothers which receive the AgNPs dose before implantation, and may be later to dysfunctions of CNS ; (Simkó and Olof Mattsson, 2014) revealed that engineering nanomaterial's (ENM) can pass the blood brain barrier (BBB) and accumulate within the brain. (Song *et al*., 2015) suggested the animals were exposed to TiO2 NPs; the NPs could be translocate into the brain mainly through (BBB) or nose brain pathway. As well as, NPs may affect the brain development of embryo by crossing the placental barrier. These nanoparticles can be possess through the BBB may be results this crossing occurred before or during BBB formation in embryogenesis.

The BBB is a well protective structure, which is mainly composed of endothelial cells, astrocytes, and pericytes, in another word, BBB is capable of protecting the healthy and functional CNS from being affected by harmful chemicals, toxins, and drugs in the circulatory system, so any damage of this barrier respectively lead to brain tissue damage (Ballabh *et al*., 2004) and (Song *et al*., 2015).

Our histological results revealed AgNPs could be enter to the embryo's brain and showed many histopathological defects in brain tissue at the higher dose of the AgNPs (20mg kg/B.wt) than the lower dose this may be due to NPs could not only pass through the BBB but also disrupt the integrity of the BBB. On the other hand, brain damage during brain development may be results of placental barrier disruption and (Semmler-Behnke *et al*., 2014). The histological results revealed apoptosis, degenerative and other effects on neurons and glial cell in cerebellum cortex, (Prakash *et al*., 2017) showed after administration of AgNPs in pregnant Swiss Albino mice, astrocytes swelling was the most significant change observed in fetuses brain, this histopathological changes and apoptosis may be results of ROS formation. Many studies showed that (ROS) had the role of signaling molecules, could influence apoptosis and the activation of different signaling pathways. Singh and Ramarao, 2012 found that AgNPs after they entered the cell can release silver ions and free radicals, which led to an oxidative environment that can disturb the mitochondrial functions. On the other hand, AgNPs can pass into the mitochondria and alter their functions by disturbing the electron transport chain. These changes advance to higher levels of free radicals and lower concentrations of ATP (Foldbjerg *et al*., 2010), (Dănilă *et al*., 2017) previous studying of Prakash *et al*., 2017, suggested degeneration with dysmorphology of cortico-medullary layers of cerebrum, cerebellum and hippocampus of mouse fetuses when administrated in lower dose (0.5 and 1 mg/kg/day) and higher dose (10 and 15 mg/kg/day) reaction products were found accumulated outside and inside of neuronal cell membrane.

V. Conclusion

Silver nanoparticles can reaches to the fetus's blood brain barrier of brain and disruption of tight junction and more dilatation of micro porous which cause easy passage of this small size nano metal to the embryos during embryogenesis. AgNPs at low concentration 2mg/kg/day and higher concentration 20mg/kg/day can be produce many histological toxicity to the embryos hindbrain and cerebellum when administrated to the dams during pregnancy period.

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