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Iron Oxide Nanoparticles Induced Histological Alteration and Fetal Skeletal Abnormalities in the Embryo of Albino Rats

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Abstract

The study was carried out to investigate the effect of iron oxide nanoparticle(NP) on the brain, liver, spleen and vertebral colum in the embryo of albino rats at the age of 19 day of pregnancy . The study included twenty (20) embryos divided into two groups ,treated and control group . The results of the histological study reveled the existence of histological alteration in the brain, liver, spleen and vertebral colum of treated groups compared with controls .

***Keywords**: Histological Alteration, Fetal Skeletal, Abnormalities.*

Introduction

Nanotechnology is the mere controlling and invention of materials at nano scale level, where characteristics vary with different size, shape ,aggregation and surface area. This technological leads to a revolution in the field of electronics and communications, optics, chemistry, energy and biology [1]. Iron oxide nanoparticles(IONPs) have encouraging characteristics like biocompatibility and magnetic behavior that makes them perfect agents for magnetic resonance imaging, carriers for drug delivery, magnetic hyperthermia, tissue engineering, cell separation, enzyme immobilization, protein purification and biosensing [(2,3).] Moreover, IONPs offer significant improvements in water purification and environmental remediation because of low cost technology [4] . Iron and oxygen chemically combine to form iron oxides (compounds [5] . The general process of manufacturing nanoparticles include laser ablation, plasma synthesis, combustion, arc method, electrolysis, pyrolysis, diffusion flame synthesis, chemical precipitation and vapor deposition and mechanical processing, wet phase processing and high energy ball milling [6] .A recent study on mice reported size dependent bioaccumulation and transport of IONPs in the liver and spleen [7] . Pregnant mice treated with multiple doses of IONPs reported biodistribution of iron in the foetal liver, placenta and increased foetal deaths [8]

Material and Method

This study was conducted in the studying of the effect of IONPs on the brain , liver, spleen and vertebral colum in the embryo of albino rats.

Animals: Thirty six healthy adult female albino rats weight (225±10gm), age (10-12)weeks were purchased from Iraqi Center for Drug Research/ Baghdad. All these animals were housed during the period of experiment in the animal house unit in science college of Babylon University, under controlled temperature (21 ± 1 C 0) and constant light-dark schedule (12 hours light and 12 hours dark cycle), food and water were available. The pregnant rats divided into tow groups:

Group1: administrated orally (150 mg/kg) of body Wight iron oxide NPs by gavage tube for 19 days of pregnancy.

Group2: administrated orally (1ml) distilled water by gavage tube for 19 days of pregnancy.

At 20 day of pregnancy the pregnant rats were killed and the embryo extracted for histological Alteration and Fetal Skeletal Abnormalities study.

Experimental Design:

Twenty embryos divided into two groups .

Group I: Control group (n=10).

Group 2: Treated group (n=10) .

Embryos Extraction and Reservation:

After dissecting pregnant females in the 20 days of pregnancy, the uterus horns were extracted which contain embryos. Then the embryonic membranes were removed surgically by fine surgical tools and dissection microscope and the embryos were taken and washed with normal saline (Nacl 0.9%) into a petri dish. The embryos were fixed directly by Aqueous Bouin's Fixative for 24 hours. After fixation, the embryos were washed several times by ethyl alcohol (ethanol) at concentration 70 % in order to remove the yellow color from embryos [9] .

Histological Study:

After fixation, serial alcohol was used for dehydration of the tissue samples (brain, liver, spleen and embryo). Tissue specimens were cleared in xylene and embedded in paraffin. The paraffin blocks were sectioned at 5 microns thickness by microtome. The obtained tissue sections were collected on the glass slides and stained by Hematoxyline and Eosin stain for histopathological examination by the light microscope [10] .

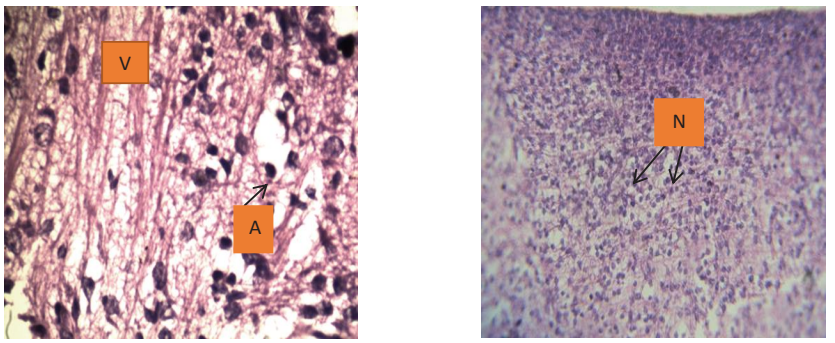
.While the vertebral colum of the embryo was prepared and stained by Alizarin stain according to [9].

Results

Histological study of the embryo

Histopathology of brain

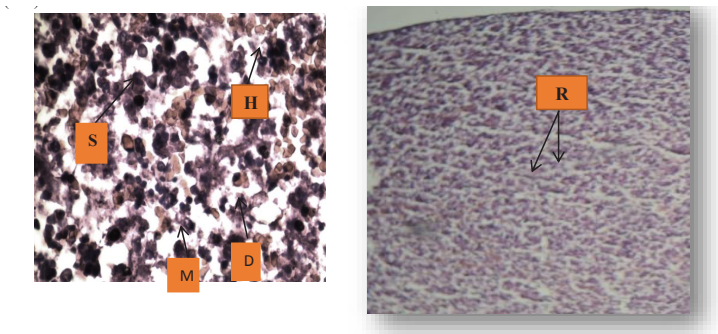
The main histopathological changes of the brain sections show observed vaculation and degeneration in the neuron cells with glial cells in the embryo at the age 19th day of pregnancy compared with control groups. Figure(1-1)



Figure(1-1): Cross section in the brain of the embryo.(A) showed sever Vocuolation in the nervous tissue(V) with astrocytes (astrocytosis)(A) (H&E 40X).(B) control group (control group) showed normal brain tissue with Normal neuron (N). (H&E 10X)

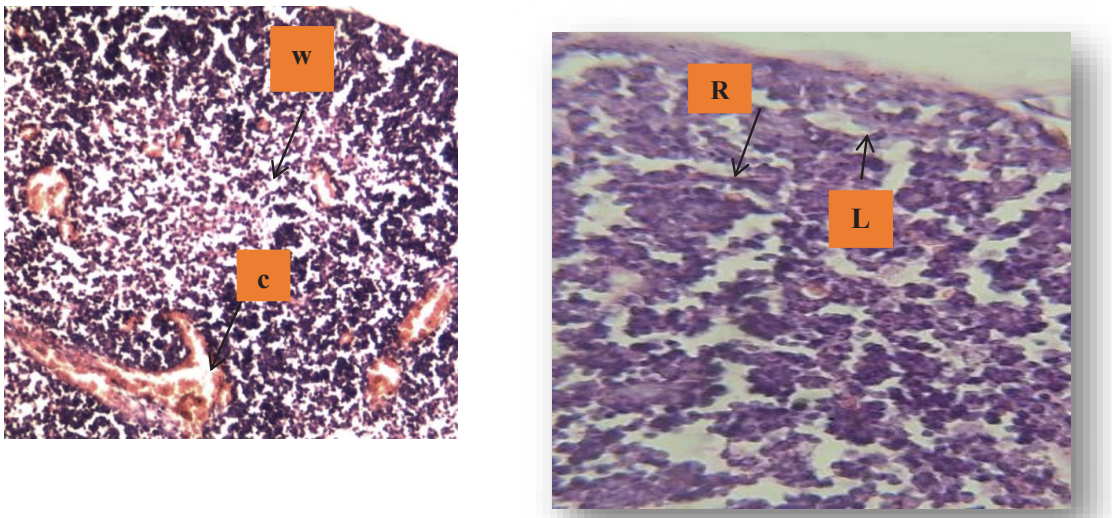
Histopathology of liver

The main histopathological changes of the liver show degeneration of hepatocytes, hemorrhage in the hepatic tissue and infiltration of inflammatory cells and dilation of sinusoids compered with control groups. Figure (1-2)



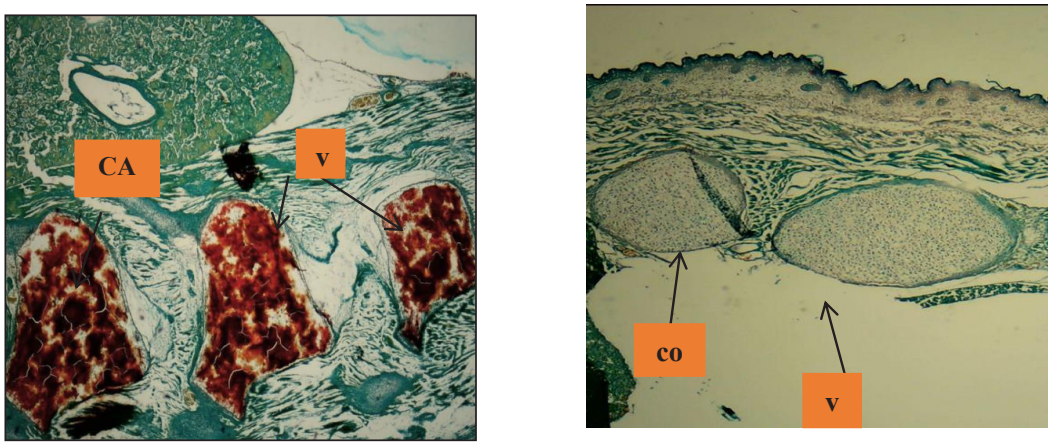
Figure(1-2): Cross section of the liver of the embryo at age 19th day of pregnancy.(A) showed Degeneration of hepatocytes(D), hemorrhage in the hepatic tissue(H) and infiltration of inflammatory cells(M) and dilation of sinusoids(S) (H&E 40X).(B) (control group) showed normal hepatic architecture(R). (H&E 10X)

The histopathological changes of the spleen sections show depletion of white pulp and proliferation of red pulp with congestion of blood vessels in the lymphoid tissue compered with control group. Figure(1-3)



Figure(1-3): Cross section of the spleen in the embryo at 19th day of pregnancy (A) showed depletion of white pulpe (W) and proliferation of red pulp with congestion of blood vessels in the lymphoid tissue (C) (H&E ,40X) .(B) (control group) showed proliferation of lymphocyte (L) and proliferation of red pulp(R). (H&E ,10X)

The histological change of skeletal malformation of embryo vertebral Colum at the age 19th day of pregnancy show abnormalities in the vertebral colum which include irregular and large vertebral with profuse calcification , coloure compered with control groups.Figure(1-3)



Figure(1-4) :Cross section in the vertebral colum of embryo at 19th day of embryo. (A) show irregular and large vertebral (V)with profuse calcification (CA).(B) (control group) show normal vertebrae (V) with normal proliferation of chondrocyte(CO) stain with Alizarin stain (10X).

Discussion

The cellular toxicity of iron oxide nanoparticle on organs were very clear in our study, NPs may cross the blood-brain barrier (BBB) and accumulate in the central nervous system (CNS), brain very important organs clinical signs in the animals its showed very hyper activity because the change in the brain tissue of treated embryo by iron oxide nanoparticle, the importance of the histological changes of the brain in the embryo the histological change of 19th day embryo include sever Vocuolation in the nervous tissuewith astrocytes (astrocytosis) compered to the control groups. These results agree with anther study who recognized that iron oxide nanoparticles may induce damages to the neural tissues as results to accumulation of the iron oxide that induce the increases in levels of oxidative stress and ROS production that induce important roles in some critical diseases such as Alzheimer's and Parkinson's [11]. Anther study confirm that IONPs accumulation in tissues triggered ROS generation which significantly altered the antioxidant enzyme levels, the altered antioxidant status and bioaccumulation of IONPs caused histo morphological changes which demonstrate that increase in oxidative stress affects the cellular structure [12] .

The defect in liver tissue is very important and in the histopathological observation showed degeneration of hepatocytes, hemorrhage in the hepatic tissue and infiltration of inflammatory cells and dilation of sinusoids. Nanoparticles are initially reabsorbed by Kupffer cells, cell membranes will be damaged and toxic degradation products of iron oxide magnetic nanoparticles will be slowly imported to hepatocytes from macrophages [13]. These findings are agreed with [4] who described damaging processes generated in the livers of rats after exposure to iron oxide nanoparticles. Another study referred that using of high concentration of iron oxide nanoparticles can caused undesirable effects in the liver with damage to the hepatocyte and elevation the level of liver enzymes, iron oxide nanoparticles (IONPs) can cause damaging in the liver-based presence of inflammation, congestion of the interstitial tissues, peri-central-vein-based fatty degeneration, and necrosis of hepatocytes [15] .

Our study demonstration important change in the spleen, In embryo of 19th day reveled depletion of white pulpe and proliferation of red pulp with congestion of blood vessels in the lymphoid tissue. These results agree with [16] who found similar effects of the nanoparticles on the spleen of mice. Treatment with Fe2O3 caused series effect in the spleen tissue include red pulp congestion and prominent white pulp in the treated rats [17].

For skeletal malformation changes, Our results inducted the presence of changes in the vertebral colum of rats embryos compared with control groups, these changes includes irregular and large vertebral with profuse calcification. Oral administration of iron oxide NPs caused some mild and severe defects in the skeletal formation (vertebral colum) in the developing fetuses. The current study indicated that the use of Alizarin Red-S was the best method to detect the osteogenesis in laboratory animals such rabbit fetus simulated that reported in mouse [18]. Moreover the present result as well as that in mouse stated that the red color of the bones was due to the high affinity of the Alizarin Red-S to the calcium ions in the bones [19] . In fact, there are some reports that show some nanoparticles can transfer through placenta and cause embryo toxicity, Iron oxide nanoparticles can accumulate in the sinusoids and hepatocytes of the fetus liver [20,21]. This is in agreement with Bourrinet who mentioned that ferumoxtran-10 had major fetal skeletal malformations in both rabbits and rats [22] . Tsay stated that bone loss has been detected after increased iron ions concentration in mice, Their results revealed dose-dependent elevation of iron content in tissue with bone composition alteration and thinning of trabecular and cortical bone accompanied by high bone resorption, this may be contributed to the increased reactive oxygen species (ROS) production [23].

Conclusion

The study included twenty (20) embryos divided into two groups ,treated and control group . The results of the histological study reveled the existence of histological alteration in the brain, liver, spleen and vertebral colum of treated groups compared with controls .

Financial Disclosure: There is no financial disclosure.

Conflict of Interest: None to declare.

Ethical Clearance: All experimental protocols were approved under the Collage of Science, Babylon University, Iraq and all experiments were carried out in accordance with approved guidelines.

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