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Histological and Anatomical Manifestations of the Effect of Chlorpyrifos Toxicity on Placenta and Fetuses of Albino Rats

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ABSTRACT

Background and Objective: Chlorpyrifos is one of the most widely used organophosphate pesticides worldwide and has been linked to harmful effects on reproductive health, however, its integrated histological and morphological effects on the placenta and fetuses of albino rats during pregnancy remain poorly understood, creating a research gap that necessitates further study.

Methods: To address this gap, pregnant albino female rats were treated with a specific dose of Chlorpyrifos for 10, 15, and 20 days. Placental tissues were histologically examined using Hematoxylin and Eosin (H&E) staining, while fetuses underwent detailed external morphological examination compared to a control group.

Results: revealed progressive histological deterioration in the placenta proportional to exposure duration. At ten days, cellular degeneration, vascular congestion, and inflammatory infiltration were observed. At fifteen day these escalated to vascular sclerosis, hemorrhage, and ischemia. By twenty day, Giant Trophoblast Cells appeared alongside extensive degeneration in the Labyrinth zone. In contrast, no visible morphological abnormalities were recorded in -fetuses across all groups, although their absence does not exclude underlying damage at the histological and molecular levels.

Conclusions: These findings confirm that Chlorpyrifos poses a real histological risk to the placental-fetal unit; and necessitate stricter monitoring of pregnant women's exposure and reassessment of its use policies in agricultural and domestic environments.

Introduction

Chemical pesticides have become an integral cornerstone of the modern agricultural system, however, what they leave behind of unexpected effects on human health has become a growing concern, especially with more vulnerable and exposed people like agricultural land workers and related sectors[1]. The danger of this pesticide is not limited to farmers, it also reaches residential complexes through its use in combating household insects and garden care [2]. The accumulated epidemiological studies have identified a diverse range of documented negative health effects connected with pesticide exposure, including disruption of neurological and psychological development, fetal abnormalities, respiratory diseases, and even certain types of cancer [1]. Among the most prominent pesticides in scientific attention are organophosphate pesticides which follow highly complex pathways in their biological effects, and most notably chlorpyrifos which is classified as one of the most widely used worldwide and utilized to eliminate ticks, insects, and termites [3]. Given what studies demonstrated that Chlorpyrifos is a neurotoxic substance that has documented effects on immune and psychological health. Despite many countries have banned or restricted Chlorpyrifos, it remains widely used in many agricultural areas around the world [4,5].The residues of this pesticide in the food chain represent one of the most prominent health concerns on a global level, particularly as its effect remains accumulated on agricultural products or deeply penetrating them for a long period following spraying, and the risks of exposure notably increased during pregnancy, as pregnant women may be exposed to this substance through drinking water, food, and herbal preparations which may lead to serious consequences affecting both mother and fetus [6]. The placenta in this context is not merely a functional organ linking the mother to her fetus, but rather the primary vital barrier against harmful external substances, and performing critical functions in regulating the transfer of nutrients and gases and secreting the necessary hormones for the continuation and safety of pregnancy [7]. Although the placenta has notable functional capacity that allows it to compensate for some toxic damage [8], exposure to Chlorpyrifos has proven capable of overcoming this compensatory capacity, as the studies demonstrated that it affects placental hormonal secretion and activates cell death receptors [6], in addition to stimulating oxidative stress, apoptosis, and placental barrier dysfunction through the MAPK/ERK signaling pathway. The study indicates that exposure to Chlorpyrifos in the early stages of pregnancy may be a potential risk factor affecting placental formation and its functions in both humans and animals [9]. On the level of fetal effects, pregnant women's exposure to Chlorpyrifos has been associated with reduced birth weight, impaired reflexes, decreased intelligence quotient (IQ), and increased rates of pervasive developmental disorders [10]. More recent studies reported that the experimental dietary exposure to Chlorpyrifos causes disruptions of the placental immune system at the genetic level [11], At the molecular level, prenatal exposure to Chlorpyrifos in Trophoblast-like cells has been shown to alter the expression of genes necessary for placental development, potentially impairing its function and affecting DNA methylation levels and cognitive performance in the offspring [12]. Detailed histological studies also revealed that the increase in the number of glycogen Trophoblast cells and giant cells in the labyrinth and junctional zones is considered an indicator of histological impairment associated with hypoperfusion and placental vascular dysfunction [13]. Despite the accumulating evidence of Chlorpyrifos toxicity in multiple animal models, such as fish, birds, and crustaceans, its chronic and integrated effects on placenta and fetal development in the albino rat model remain poorly understood. From this research gap, the urgent need for comprehensive histological and anatomical studies to elucidate the precise mechanisms by which this pesticide affects the placental-fetal unit during pregnancy emerges. Accordingly, the present study aims to evaluate the histological and morphological effects resulting from Chlorpyrifos exposure on the placenta and fetuses during pregnancy.

Materials and Methods

This experimental study was conducted in the animal laboratory unit of the College of Veterinary Medicine / University of Tikrit during the period of two months from October to December of 2025. The experiment included 20 female albino rats aged between 12 and 14 weeks and weighing between 190 and 250 grams. They had been randomly divided into four equal groups with 5 animals in every group, and were put in standard plastic cages sterilized with 70% ethanol, and were left for 10 days to adapt with the laboratory environment. Mating for female albino rats was performed by gathering them in a large cage with appropriate number of adult males of the same strain. After examination of the the female rats to insure the presence of vaginal plugs

as evidence of successful fertilisation, the fertilised females were returned to their individual cages and were considered in day zero of pregnancy and left untreated for 24 hours, then pesticide treatment of animals was started. The animals were sprayed once daily with Chlorpyrifos 48%, which was diluted by mixing 1 ml of the pesticide with 50 ml of distilled water, the dose (4 ml, in the form of 8 sprays) was calculated based on the body weight of the animal and the surface area of the cage, so that 4 sprays were aimed at the sides of the cage and 4 sprays were aimed directly on the animals from the distance of 22 cm. The procedure was conducted inside a closed spraying box in accordance with the safety protocols. After spraying, the animals were left for one hour to monitor the clinical signs and behavioral changes, such as aggression and social withdrawal. The procedure was repeated in the same way throughout the experiment's period. [14], the exposure period varied between the three groups to be: 10, 15, and 20 days respectively until the end of the experiment, and on day 21 of pregnancy, before parturition, all the animals were anesthetized and dissected, as fetuses and placental tissue samples were collected from the four groups. The fetuses went under a detailed systematic external morphological examination, while the placental tissues were fixed in 10% formalin, then processed into thin histological sections, and stained with Hematoxylin and Eosin (H&E) for microscopic examination.

Results & Discussion

Control group

The histological examination of placental tissue in the control group showed a well-developed normal structure. The sections were characterized by an intact labyrinth zone with normal maternal blood spaces, syncytiotrophoblast cells with normal appearance, and a clear junctional zone with structurally intact fetal blood vessels, as shown in Fig. 1.

(10 days) group

In the 10-day group, the histological examination revealed the start of clear pathological changes, as multiple focal cellular degenerations were noticed, accompanied by prominent vascular congestion and ruptures in cytotrophoblast cells, alongside infiltration of inflammatory cells in various regions, and loss of parts of the labyrinth zone and degeneration of maternal blood spaces, indicating the beginning of placental functional deterioration, as shown in Fig.2.

(15 days) group

With the extension of the exposure period to 15 days, the intensity of pathological changes increased notably, as the histological sections showed blood vessels with thickened walls indicating clear vascular sclerosis, hemorrhage in various regions, histological deterioration reflecting ischemia, in addition to the deterioration of syncytiotrophoblast and trophoblast cells, and a widespread inflammatory cellular infiltration, as shown in Fig. 3.

(20 days) group

Histological deterioration reached its peak in the twenty-day group which represented the most severe stage of pathological change, as histological sections were characterized by the appearance of Giant Trophoblast Cells in a notable density, accompanied by extensive degeneration in the labyrinth zone and a severe inflammatory infiltration, as shown in Fig. 4.

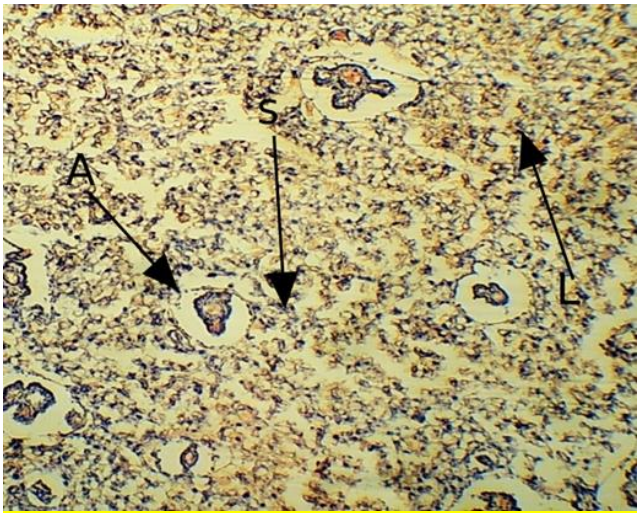

Fig. 1. Cross-section of rat placenta in the control group showing a normal histological structure: Labyrinth zone(L), maternal blood spaces(A), and Syncytiotrophoblast cells(S), H&E stain, ×10

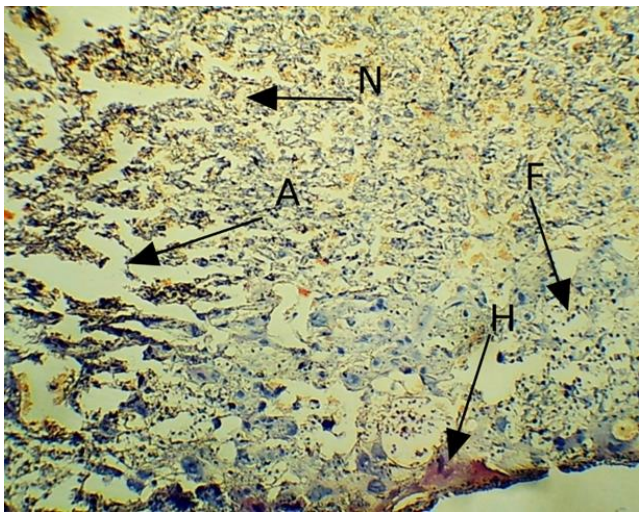

Fig. 2. Cross-section of rat placenta in the 10-day treated group showing: early pathological changes, cellular degeneration(N), vascular congestion(H), ruptures in cytotrophoblast cells(A), and infiltration of inflammatory cells(F), H&E stain, ×10

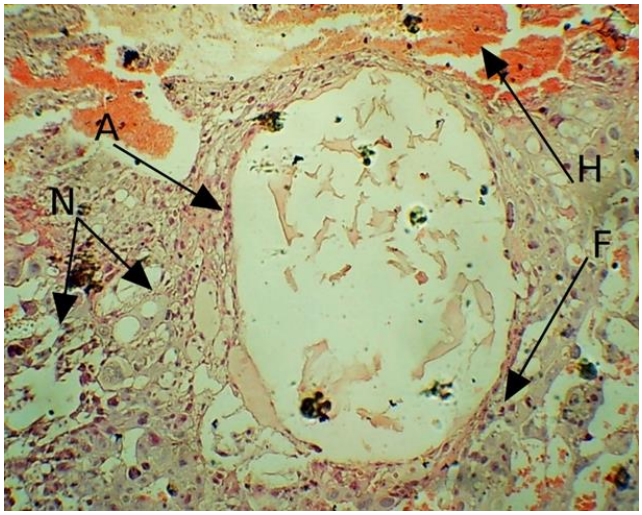

Fig. 3. Cross-section of rat placenta in the 15-day treated group showing: Blood vessel with thickened walls indicating vascular sclerosis(A), tissue degeneration surrounding the vessel(N), inflammatory cell infiltration(F), and hemorrhage(H),H&E stain, ×40

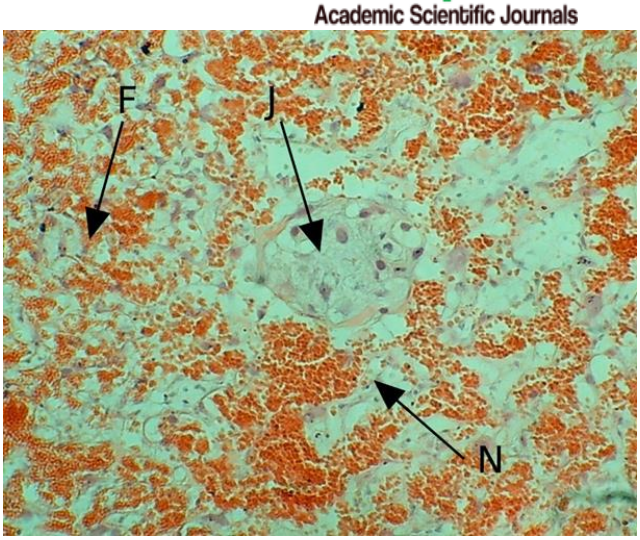

Fig. 4. Cross-section of rat placenta in the 20-day treated group showing: Giant trophoblast cells(J), cellular degeneration(N), and severe inflammatory cell infiltration(F), H&E stain, ×40

Histologically, regarding the control group, the results are consistent with what a study documented, that the labyrinth zone in the placenta of rodents represents the main site of oxygen and nutrients exchange between the mother and fetus, and the integrity of this zone and the syncytiotrophoblast cells connected with it is considered the reference histological standard of a functionally normal placenta [15].

Regarding the 10-day group, the results are consistent with what a study confirmed that CPF induces a notable increase in reactive oxygen species (ROS) in placental trophoblast cells, leading to placental barrier dysfunction through activation of the MAPK/ERK signaling pathway, which initiates a cascade of mitochondrial apoptotic reactions [9]. This interpretation also supports what a group of researchers observed that exposure to CPF through dietary exposure causes disruptions in the placental immune system at the genetic level, which explains the early inflammatory infiltration observed at this stage [11].

The histological pattern in the 15-day group is consistent with what a study reported that prolonged exposure to CPF causes an extensive disruption in the proteins forming the intercellular junctions between the placental epithelial cells, resulting into a complete functional disruption of the placental barrier [9]. This result reinforces what a study observed in their comprehensive systematic review that CPF passes the placental barrier easily, due to its hydrophobic nature (Log Kow = 4.7), accumulating in adipose tissues causing multifaceted toxic effects [16].

A group of researchers has revealed in their detailed study of damaged placenta that the increase in numbers of glycogenic trophoblast and giant cells in the labyrinth and junctional zones represents an indicator of histological impairment associated with hypoperfusion and placental vascular dysfunction, which is consistent with the compensatory response resulting from accumulation of CPF-caused damage in the present study regarding the 20-day group [13]. In this context, a study has confirmed that prolonged exposure to CPF reduces the expression of proteins regulating intercellular communication in the placenta, which disrupt regulation of trophoblast cell proliferation and differentiation [9], and that mechanisms of CPF toxicity on the placenta are not limited to cholinergic inhibition but extend to include endocrine disruption and modulation of gene expression via epigenetic modifications [16].

Morphological results of the fetuses

The detailed external examination of all studied groups revealed a complete absence of apparent morphological abnormalities; as fetuses were in normal range in terms of general size, body length, symmetry of anterior and posterior limbs, longitudinal body axis, and head development, and free of any signs of hemorrhage, edema, or cranial abnormalities, as shown in Fig.5.

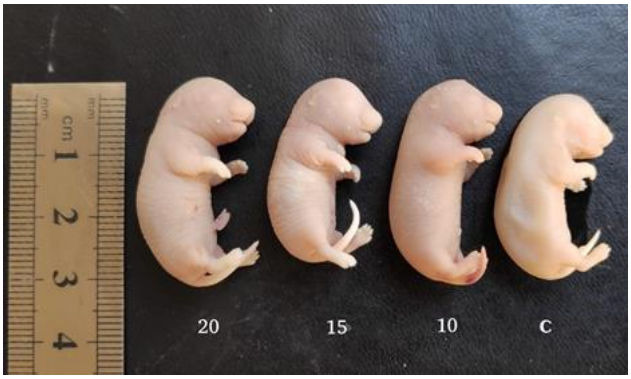

Fig. 5. Lateral view of Albino rat fetuses at the end of the experimental period, showing a comparison between the control group(C) and groups treated with Chlorpyrifos for different durations (10,15, and 20 days), all fetuses appear within normal limits in terms of external morphological characteristics.

Morphologically, this result is consistent with what a study demonstrated that oral exposure of female rats to Chlorpyrifos in sub-maternal toxic doses did not produce apparent morphological abnormalities [17], and with what another study concluded that major teratogenic effects occur primarily at doses inducing overt maternal toxicity, while the external abnormalities remain absent or scarce at doses below that threshold [16]. However, the absence of apparent abnormalities does not necessarily mean complete fetal safety, but reflects a genuine limitation in the superficial morphological examination capacity to detect the latent damages forming at the histological, molecular, and neurological levels. A study revealed severe structural abnormalities in domestic chicken embryos when exposed to a sublethal dose of a mixture of Chlorpyrifos and Cypermethrin, including early somite defects, limb abnormalities, and cartilage structure deformities, confirming that the pesticide is causing teratogenic effects in some animal models [18]. However, these effects vary considerably between species. As a study demonstrated that the combined exposure of the two pesticides in zebrafish caused abnormalities in cranial cartilage that did not appear in the individual exposure indicating that the teratogenic toxicity may be conditioned by additional factors such as synergism between substances and the timing of exposure, in addition to the abnormalities in the non-mammalian models cannot be directly extrapolated to rodent models due to essential differences in the placental structure and the metabolic detoxification efficiency [19].

The interpretation for the absence of apparent abnormalities in the present study supports that the accumulated pathological changes in placental tissue, even if they gaining a progressive character with the extension of exposure period, they did not reach the sufficient threshold to disrupt the regulatory processes of axial patterning and organ guidance during the embryonic organogenesis stage, the present study also confirms that the documented histological abnormalities in the placenta including vascular congestion, degeneration in epithelial trophoblast cells, impairment of the labyrinth zone, and inflammatory infiltration may cause deficiency in nutritional exchange and oxygen supply without necessarily resulting in apparent abnormalities, which is consistent with what a study concluded that the exposure to low doses of organophosphate pesticides during pregnancy may cause disruptions in the maternal hypothalamic-pituitary-gonadal axis, with the possibility of transmitting these effects to the fetus through the placenta in subtle ways that leave no apparent external trace in the early embryonic stage[20],

suggesting that the latent damage of histological, molecular, and hormonal nature documented in this study represent the actual foundation of the cumulative harmful effects of the pesticide which may manifest in later stages of development, increased dosage, or advancement of the exposure period.

Conclusions

The present study demonstrated that exposure to Chlorpyrifos during pregnancy causes progressive and cumulative histological deterioration in placental tissues directly proportional to the duration of exposure, as the pathological changes advanced from cellular degeneration, vascular congestion, and inflammatory infiltration in the ten-day stage, through vascular sclerosis, hemorrhage, ischemia in the fifteen-day stage, reaching the appearance of the Giant Trophoblast Cells and extensive degeneration in the labyrinth zone in the twenty-day stage, reflecting advanced disruption in the placental barrier function and the integrity of the placental-fetal unit. In contrast, Chlorpyrifos's treatment did not result in any apparent fetal morphological abnormalities across all treated groups, as the fetuses fell within normal limits in terms of size, shape, and symmetry of limbs. However, the absence of apparent abnormalities does not necessarily mean complete fetal safety, suggesting that latent damage of histological, molecular, and hormonal nature represents the actual foundation of the cumulative harmful effects of the pesticide, which may manifest in later stages of development or with increased duration of exposure or dosage.

Recommendations

1. Regulatory policies concerning Chlorpyrifos use in both agricultural and domestic environments should be reassessed, with setting stricter exposure limits for pregnant women, given the clear histological damage observed in the placenta even in the absence of apperent fetal abnormalities.

2. Future studies should be expanded in assessing the fetuses beyond external morphological examination, to include histological, molecular, and neurodevelopmental evaluation, as this study showed that damage may remain latent and undetectable through morphological examination alone.

3. More researchs should be conducted to determine a safe dose, and evaluate the long-term effects on the newborns after birth, taking in consideration the clear correlation that this study detected between the duration of exposure and the severity of placental damage.

Acknowledgement

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Ethics Statement

All experimental procedures involving animals were conducted in accordance with ethical guidelines and approved by the Institutional Ethics Committee of the University of Samarra (Approval No. EDUSamarraA01/2025/005).

Declaration of interests

The authors declare that there is no competing interest.

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المظاهر النسيجية والتشريحية لتأثير سمية الكلوربيريفوس على المشيمة والأجنة في الجرذان البيضاء

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الملخص

الخلفية والهدف: يعد الكلوربيريفوس أحد أكثر مبيدات الفوسفات العضوية استخداماً على مستوى العالم وقد ارتبط بأثار ضارة على الصحة الانجابية، غير أن لاتزال أثاره النسيجية والمظهرية المتكاملة على مشيمة وأجنة الجرذان البيضاء أثناء الحمل غير مفهومة بالقدر الكافي، مما يشكل فجوة بحثية تستدعي مزيداً من الدراسة لسد هذه الفجوة.

المواد وطرائق العمل: عوملت إناث الجرذان البيضاء الحوامل بجرعة محددة من الكلوربيريفوس لمدة 10, 15, 20 يوماً، بعدها خضعت انسجة المشيمة للفحص النسيجي باستخدام صبغة الهيماتوكسيلين والإيوسين (E&H)، فيما خضعت الأجنة للفحص المظهري الخارجي الدقيق مقارنة بالمجموعة الضابطة.

النتائج: كشفت الفحوصات عن تدهور نسيجي تدريجي في المشيمة يتناسب مع مدة التعرض، حيث لوحظ في مجموعة العشر ايام تنكس خلوي وإحتقان وعائي وتسلل التهابي، بينما في مجموعة الخمسة عشر يوماً تفاقمت هذه الأعراض إلى تصلب وعائي و نزيف و نقص تروية، اما في مجموعة العشرين يوماً فقد ظهرت خلايا الأرومة المغذية العملاقة مصحوبة بتدهور واسع النطاق في منطقة المتاهة، في المقابل لم تُسجل اي تشوهات مظهرية في الأجنة لجميع مجاميع الدراسة، مع أن غيابها لا ينفي وجود ضرر كامن على المستويين النسيجي و الجزيئي.

الاستنتاجات: تؤكد هذه النتائج أن الكلوربيريفوس يشكل خطراً نسيجياً حقيقياً على وحدة المشيمة والجنين، مما يستدعي مراقبة أكثر صرامة لتعرض النساء الحوامل له، و إعادة تقييم سياسات استخدامه في البيئات الزراعية و المنزلية.

الكلمات المفتاحية: الكلوربيريفوس، المشيمة، الأجنة، السمية التطورية، علم الأنسجة المرضية.