Using Histopathological Change as Bioindicator for Hydrocarbon Toxicity in Chick Embryo

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Abstract:

Histopathology studies the microscopic alteration of cells in tissue organs of living beings. Because of their economic significance and ability to be used as a model to predict the sensitivity of wild bird species to chemical contamination, chickens were chosen for this study. The present study was carried into control and treated chick embryos with two concentrations of hydrocarbon wastes for tissue organs (liver and brain). The results showed that PAHs have pathological effects represented by hydropic degenerative changes, necrotic tissue area, and congestion of blood vessels in the liver and brain. The pathological changes have been severed in group B (4ppm/egg of PAHs) compared with group A (2ppm/egg of PAHs). These results suggest that chick embryo liver and brain are sensitive to hydrocarbon's adverse effects.

Keywords: histopathological changes, hydrocarbon compound, chick embryo

1- Introduction

The main way that chemicals from hazardous waste storage facilities are exposed to humans and animals is through water (1). The main causes of groundwater pollution are hazardous waste sites (HWS), municipal waste, industrial waste, leaching of minerals from natural deposits, unintentional spills and leaks, and agricultural operations (2,3).

These HWS have been identified by the U.S. Environmental Protection Agency (EPA) as one of the main hazards to the environment and living things (4). The Council for Agricultural Science and Technology (5) reports that 85% of Americans living in rural areas and 50% of the country's total population rely on groundwater as their primary source of drinking water. The level of human or animal exposure to chemical waste and the ensuing health issues are among the few details that are currently accessible regarding the health effects of groundwater toxins. The International Agency for Research on Cancer states that certain PAHs are known to have the potential to cause cancer in people (6). Widespread fused-ring pollutants known as polycyclic aromatic hydrocarbons (PAHs) are created when practically all organic materials, whether natural or man-made, burn incompletely. Numerous PAHs are present in animals worldwide, including bird species, and some have been demonstrated to cause cancer in people. Numerous studies have looked at the toxicological effects of exposure to PAHs in a variety of taxa, including fish, reptiles, mammals, microbes, and invertebrates, with confirmed effects on toxicity and tumor formation, among others (7-14) demonstrate how challenging it is to assess how water pollution affects the chicken industry. A survey of the literature found little data regarding how water contaminants affect hens' ability to reproduce and the quality of their eggs. It has been estimated what the maximum safe quantities of chemicals in poultry drinking water are. However, more research is required to determine how accurate these figures are. For both Na and Cl, the maximum acceptable amounts in drinking water were 1,000 and 1,500 mg/L, respectively (15,16)

found that chickens fed water enriched with 2,000 mg NaCl/L (41 and 35 mg/L of Na and Cl, respectively) had increased rates of embryonic death, damaged eggshells, and decreased hatchability compared to hens fed control water (17) discovered a correlation between the incidence of fatty liver syndrome in laying hens and higher water hardness and amounts of calcium, magnesium, strontium, salt, iron, and barium. Nevertheless, subsequent research revealed that elevated levels of calcium and magnesium (100 and 50 mg/L, respectively) in drinking water did not cause alterations in liver composition, body weight, or egg production (18). It is clear that if a chemical is ingested more than the recommended dosage, it may damage an animal's cells. Variability in chemical susceptibility is seen in different tissues and cells. The concentration and potentiality of the poisonous substance determine how severe the tissue damage is (19). Microscopic analyses of the tissues can reveal the histopathological alterations brought on by chemical toxicity, which also indicates the degree of tissue specificity to the chemical action. Histological examination provides a clear image of how the medications harm the tissue. Histopathology is the study of disease appearance through microscopic analysis of tissue (20). The study's objective is to use histopathology diagnosis to assess the impact of refinery waste's hydrocarbons on chick embryos.

2-Material and method

A-Experimental design:

Sixty fertile eggs were from the local market and then divided into 3 groups 20 eggs for each with 10 replicates to each group, group A treatment with a single dose of 2ppm/egg of PAHs, group B treated with a single dose 4ppm/egg of PAHs injected in the air sac (21) with 15th day of incubation period, group C dosage with tap water without PAHs and served as control group, Then injected eggs was return to humidified incubator until complete the normal incubation period (22) days. We used 70% ethanol alcohol to sterilize all of the eggs on the 19th, and the eggshell was cracked open to reveal the air sac. All of the test samples were injected straight into the inner shell, and the opening was sealed with wax to keep the embryos clean until the day of the examination. At the end of the experiment, the embryos were dissected to take samples from liver and brain organs for histopathological diagnosis study.

b- Chemicals:

A method outlined in (1) was modified to create two stock solutions. The combined combination from the west of the AL-Dura refinery was dissolved in olive oil and included in the aqueous stock solution.

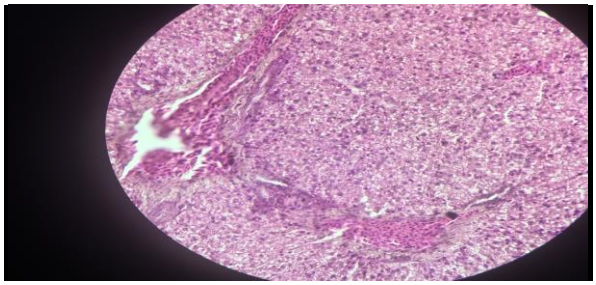
C- Tissue sampling:

Samples of tissue organs from the liver, and brain were collected from each chick embryo was preserved in a concentration of 10% formalin solution. After 48 hours passed in the ascending concentration of ethanol solution (70%, 80%, 90%, 100%) respectively then in xylem and paraffin wax embedding in cast of paraffin wax then cut to slices of 4-7 micron thickening and stained by routine stain hematoxylin and eosin then covered with cover slip then diagnosed microscopically.

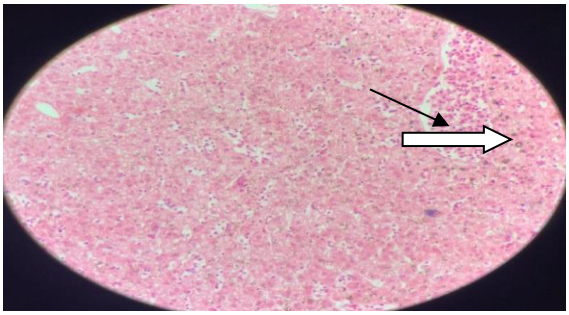
3-Results and discussion

The results of histopathological changes for exposed groups (A and B) compared with control represented by the following:

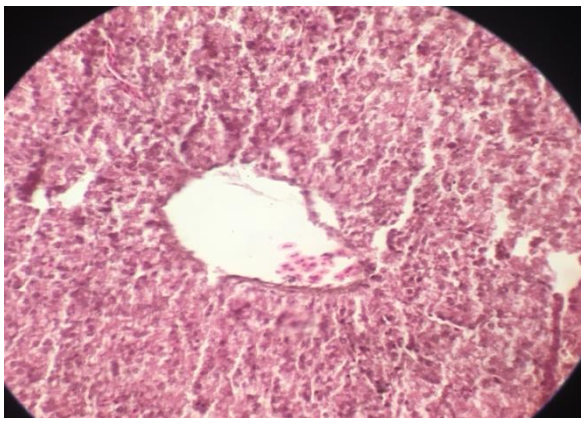
Organ	Stock A	Stock B	Control
liver	Some necrotic tissue areas, infiltration of inflammatory cells, hydropic degeneration congestion, and widening of central vein and present oedematous fluid with perivascular infiltration of inflammatory cells.	Central vein congestion, perivascular necrosis, inflammatory cell infiltration, and hydropic degenerative alterations widening the central vein, presence of focal necrosis also single cell necrosis (Apoptosis). perivascular proliferation of fibroblasts	No histopathological changes seen
Brain	Congestion and widening of blood vessels, perineuronal vascular, degenerative changes pyknotic changes of a nucleus of some neoron.	Congestion of blood vessels, vasculation, and some glial cell necrosis	No histopathological changes seen



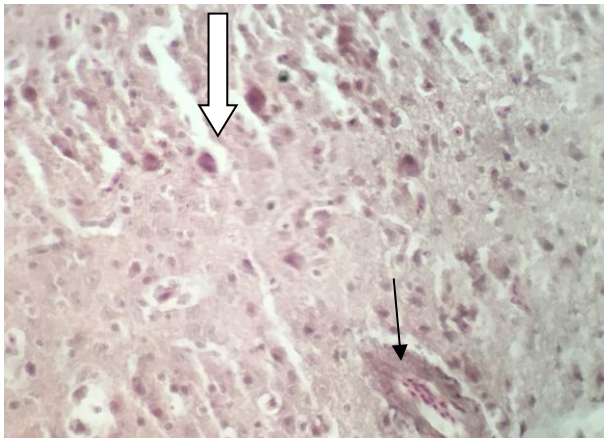
Photograph (1) liver of stock A refer infiltration of inflammatory cells, hydropic degeneration refer to oedematous fluid and present red blood cell lysis with perivascular infiltration of inflammatory cells refer to focal necrosis and karyorrhexis of nuclei



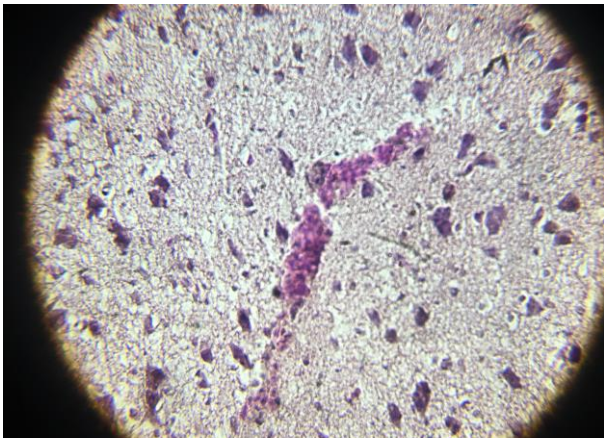
Photograph (2) liver of stock A → refer to congestion of the central vein and ⇨ refer to intravascular with perivascular infiltration of inflammatory cells.



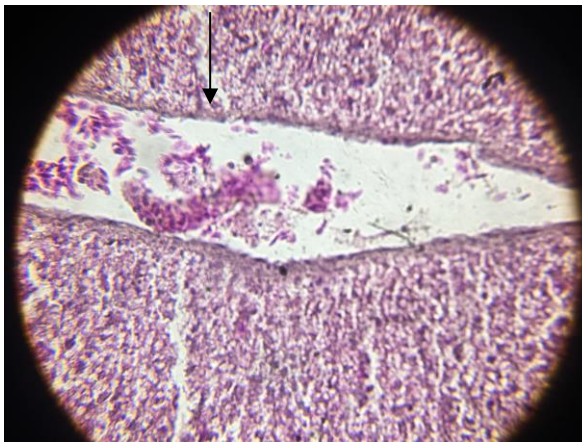
Photograph (3) show Normal tissue section of liver



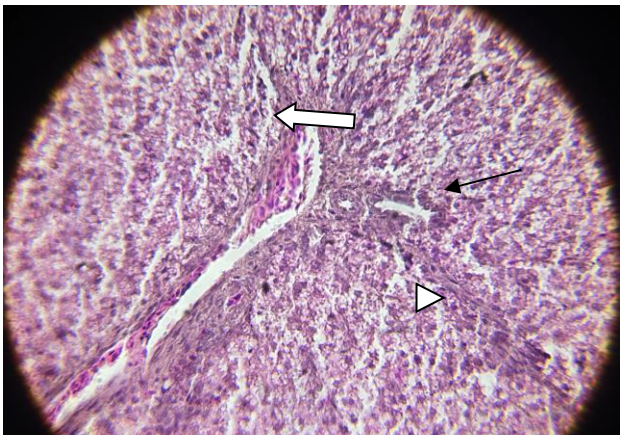
Photograph (4) brain of stock Ashow ← hyperemia and widening of blood vessels Show ⇨ peri neuronal, degenerative changes pyknotic changes of a nucleus of some neuron.



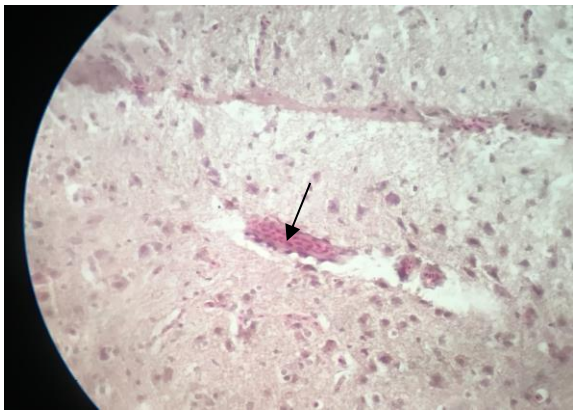
Photograph (5) show Normal tissue section of brain



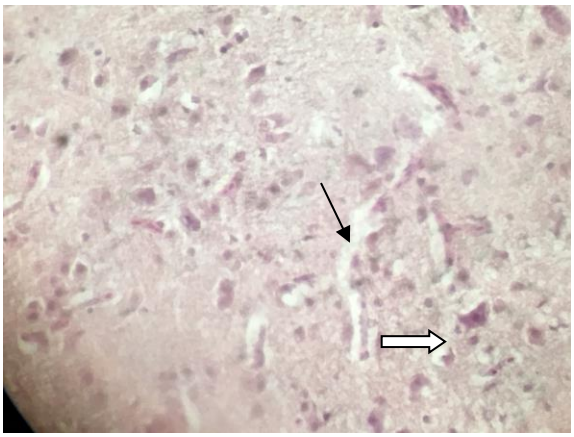
Photograph (6) liver of stock B → show Central vein congestion, perivascular necrosis, inflammatory cell infiltration, and hydropic degenerative alterations



Photograph (7) liver of stock B → shows necrotic tissue area surrounding the central vein, infiltration of inflammatory cells ⇨ and show proliferation of fibroblasts, and single-cell △ necrosis (Apoptosis)



Photograph (8) brain of stock B show ⟶ Congestion of blood vessels



Photograph (9) brain of stock B ⟶ show vasculation and ⇨ show some glial cell necrosis

Since the liver is the primary organ that metabolizes and detoxifies a variety of medications and xenobiotics, chick embryos have been used for many years to study the effects of environmental toxins. The cytotoxicity of hydrocarbons in the chick embryonic system is being studied in animals, and it depends on time and dose. To examine the indications of development, a histological section of the experimental groups' liver and brain tissues has been examined.

oxidative harm brought on by the toxicity of hydrocarbons. After injecting chick embryos, Groups A and B exhibit mild hydropic degeneration, focal areas of necrosis, karyorrhexis of the nucleus, and focal infiltration of inflammatory cells in the liver tissue, as shown in photographs 1, 2, 6, and 7. These findings are consistent with [22,23], who noted chronic hepatic inflammation in rats [24 -25], who reported that Acrylamide treatment in the rats' livers revealed frequent necrosis, pycnotic nuclei, proliferation of sinusoidal bile ducts, and hemorrhages. When cadmium was administered to rabbits, fatty infiltration was observed in their livers (26), after being exposed in ovo to the 16 PAHs mixture, the hepatic β-oxidation was lower than the control. Although the exact processes underlying the reduced fatty acid oxidation in this study are unknown, they could be brought on by complex pathways linked to endocrine disturbance, inadequate membrane structure, or the activity of enzymes regulating the rate at which fatty acids enter the mitochondria. It has been noted that PAHs cause a decrease in the hepatic β-oxidation of fatty acids in avian embryos. The current study's findings of vacuolation and liver tissue damage are consistent with (27), where the developing chick embryos injected with acrylamide showed more significant hepatocyte vacuolation around the central vein. These findings suggested that exposure to BPA gradually increased the severity of these degenerative alterations, including hydropic degeneration with a focal array of hepatic cords and increased dilatation and congestion of sinusoids and central vein. The findings concur with (28) who defined hepatocyte vacuolation as ballooning degeneration and regarded it as a type of cellular defense mechanism against harmful chemicals BPA on day 14. (29) revealed histological alterations in the liver, including hydropic degeneration, necrosis, and inflammatory aggregation, as well as sinusoidal

dilatation and blood vessel congestion. The current investigation indicates that the histopathological changes in group B are more severe than those in group A. As in photographs (4, 8, 9), the alterations in brain tissue of groups A and B are depicted by hyperemic and enlarging blood vessels, and perineuronal, degenerative, and pyknotic modifications of the nucleus of certain neurons. contrast with the control group, as shown in picture (5). Similar results were also reported by (30) demonstrating that BPA therapy produced comparable results., BPA-treated chick embryos' brains displayed demyelinating hyperemia and degenerative changes (31,32) Mortalities in chicken embryos reported for each group likewise showed a dose-dependent association. This demonstrates the obvious impact of oil waste on liver and kidney function as well as the ineffective handling of these wastes before their release into the river (33) employing fish erythrocytes' micronucleus test as a reasonable indicator for determining the level of pollution in aquatic environments (34) concluded that anthracene is poisonous to the common carp's kidney and liver.

5. Conclusion

However, our investigation has shown that these two concentrations are harming the embryos. Hydrocarbon had a more detrimental effect on the liver and brain organs of chick embryos at concentrations of 4 ppm than concentration A (2 ppm). Thus, our research, which is based on a variety of environmental compound refinery wastes, indicates that exposure to embryonic systems, particularly pregnant ones, is required to prevent damage from chemicals that are recognized during the embryonic growth stage.

Conflict of interest: None

Funding: None

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استخدام التغيرات المرضية النسجية كمؤشرحيوي للتسمم بالهيدروكاربونات في اجنة الدجاج

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وزارة التعليم العالي والبحث العلمي/ هيئة البحث العلمي

الخلاصة

يعنى علم الانسجة المرضيةبدراسة التغيرات المجهرية التي تحدث في خلاياانسجة اعضاء الكائن الحي. واختيرت الدواجن للدراسة لاهميتها الاقتصادية وكموديل لتقييم حساسية انواع الطيور البرية للتلوث بخليط من الكيمياويات . اجريت الدراسة الحالية على اجنة دواجن مجموعة السيطرة ومجموعة المعالجة بتركيزين من مخلفات الهيدروكاربونلانسجة اعضاء(الكبد والدماغ) حيث تعتبر الهيدروكاربونات الحلقية ملوثات على نطاق واسع تتكون اثناء الاحتراق الغير تام لكل انواع المواد العضوية من مصادر طبيعية وصناعية ويعتقد ان بعض مركبات الهيدروكاربون المتعددة الحلقات مسرطنة للانسان. بينت النتائج ان للهيدروكاربونات تأثيرات مرضية تمثلت بتنكس استسقائي وتنخر في خلايا انسجة الكبد والدماغ واحتقان في الاوعية الدموية وكانت التغيرات شديدة في التركيزBمقارنة مع التركيزAوتشير النتائج الى حساسية اعضاء الكبد, الدماغ, المعدة والقلب في اجنة الدجاج للتأثيرات العكسية للهايدروكاربون .

الكلمات المفتاحية : التغيرات المرضية النسجية , مركبات الهيدروكاربون , اجنة الدجاج