Study the Effect of Poly Aromatic Hydrocharbons by Using Biochemical Tests in Chicken Embryos

Sarab,S.Kadhum[1], Sarab,R.Mustafa[1] , Suha, A.Ali[2], Maha, M.taen[2], Melad.A Husien[2], Estabraq, N, Abdullateef[2]

[1]Senior Researcher, [2]Researcher, Ministry of Science and Technology/Environment and Water Directorate-Baghdad/Iraq

Abstract

The major risk of polycyclic aromatic hydrocarbons (PAHs) in all ecosystems originates from human race activity such as petroleum refining and other industrial processes. PAHs cause several effects at low doses in animals in different ecosystem. The aim of this study was to assess the treatment of AL-Dura refinery wastes before being discharged to the river by studying their effects on the liver and renal functions in chicken embryos which used as a model exposed *in vivo*. One hundred and twenty local eggs were incubated horizontally at 37.5±0.5˚C with a relative humidity of 65% in an egg incubator. Sixty eggs were used for hatchability study, were as other sixty eggs were used for biochemical analysis. On day 15th of incubation, the eggs were randomly divided into three different treatment groups and marked for identification. The first A and second group B were exposed by injections with the residues of AL-Dura refinery (after treatment) and at the concentration of (2, 4) ppm respectively and 0.2 ml / egg into the air cell with sterile siring, then sealed with melted paraffin. Control group was injected with Olive oil. On day 18th of incubation the samples of blood were collected from embryos. Liver function was measured using a biochemical assay with diagnostic kits of specific enzymes (ALT, AST, and ALP). Ceratinine and urea level were measured to asses renal function. The result showed a signenificant increase p≥ .05in the level of liver enzymes (ALT, AST), ceratinine and urea by increasing the exposure dose, were as there is a decrease in the level of ALP with the increasing of exposure dose. Hatchability rat were significantly ($p < 0.05$) decreased with dose exposure increase. Mortalities in the chicken embryos of all groups showed dose-dependent relationship. That shows there are a clear effect of oil waste on liver and renal function, and an indication of the inefficient treatment of these waste before being discharged to the river.

Keyword: *PAHs, chicken embryos, ALT, AST, ALP.*

Introduction

PAHs are well known to be toxic to avian [1] .Any toxic substances in nature can cause different changes in organisms include cellular, morphological, teratological and biochemical. When animals exposure to PAH at low doses that causes several effects related with immune and metabolic effects[2].

There is a comprehensive studies addressed the effects of PAHs on adult animals, other wise few literatures have describe the effects of PAHs on embryonic and first life stage in animals. These early developmental stages require less volume test and space that allowed using large numbers of test organisms and replicates and provide powerful statistical analysis of the results besides their sensitivity [3] .Toxicity tests with early live stage of organisms are often used to assess the toxic potential of substances and environmental samples.

For this reason, the chick embryo model is an excellent alternative to the much more expensive mammals for toxicological studies, and it is a useful tool when a study on the target species is difficult or inapplicable [4].

Chick embryos were used to detect the teratogenicity effects when exposed to BPA during sensitive periods in organogenesis induces oxidative damage and biological membranes and cellular structures damage of the brain,

and kidney, of chicken embryos [5].

Other study demonstrates the sensitivity of the chicken's reproductive system to adverse effects of contaminants commonly found in ground water [6]. Also Arleen [7] found mixed-function oxidase induction due to PAH_S in chick embryos relation with dose-response.

Liver is considered as a main target organ of environmental pollutants and any foreign substances or xenobiotics. [8] The highest capacity of metabolism is present in the liver. The level of liver enzymes in the blood was studied as an indication of dysfunction or damage in the body. The enzyme systems that metabolize PAHs are widely separated in the cells and tissues of humans and animals. For example, liver transaminases (ALT, AST) enzymes are indicators of cell necrosis in the liver tissue .Other researchers were mentioned that injection of BaP and other PAHs intraperitoneal or subcutaneous during the first 15 days of life, are caused liver and lung tumors within half a year [9].

Thus the current study was designed to evaluate the treatment of Al-Dura refinery wastes before they are discharge to the river by studying their effects in chick embryos liver and kidney which used in this study as a model exposed in vivo due to its low cost, easy accessibility and their sensitivity [10].

Material and Method

- Experimental Design

One hundred and twenty fertilized eggs with the average egg-weight of 54.5± 0.5g were purchased from the local market at March of 2019 and assigned randomly into three groups of 40 eggs each for PAHs administration on 15th day.

- Experimental group

Group A: PAHs (2 PPM/egg).

Group B: PAHs (4 PPM/egg).

Control group: (Olive oil)

Eggs were incubated horizontally at 37.5±0.5˚C in an egg incubator with a relative humidity of 65%. After72 hr. of incubation, all eggs was candled to confirm their viability. Dead eggs were removed. On day 15th of incubation, each egg was sterilized with 70% ethanol and egg shell was opened to obtain access to the air cell. Groups A and B were injected by the air sac method [11], administered as single dose of 20µg and 40µg/egg of PAHs respectively. Control group received no PAHs and served as healthy control. All eggs were covered by a wax to ensure the embryo's health until sampling takes place. At embryonic day 18, the egg shell was broken at the air chamber and blood was collected for biochemical tests. Blood were collected from each group by opening the eggs at the wider end. About 0.5 ml of blood was collected from heart of each chick embryo, was dispensed in tightly sterile tubes containing anticoagulant EDTA. Serum was separated from blood by centrifugation at 3,000 R.P.M for 10 minutes at room temperature, and stored at - 20 C until required for biochemical analysis to urea, ceratinine, (ALP), (AST) and (ALT) using commercially available kits. The rest of the injected eggs (20 eggs from each group) were placed in an incubator for hatching, at 19th day of incubation, tray rotation was stopped, and eggs were put in individual baskets and incubated at 37.5 °C and 70% humidity for hatching

Chemicals:

Two stock solutions contained mixed mixture from wastes of AL-Dura refinery dissolved in olive oil were prepared by modifying a procedure described by [12]. Solutions were stored at room temperature, protected from light, until needed for egg injections

Statistical Analysis

Data including embryonic mortalities, and hatchability were calculating by compared differences in ratios in each group. Tukey's test and One-way analysis of variance was applied to assess the significance variance in the biochemical analysis. Data were significantly different from each other considered at a p value ≥ .05.

Result and Desiccation

Hatchability and mortality:

Hatchability of each group, on the fertile eggs basis, was calculated at day 21 of incubation. Increasing in the embryonic mortalities was seen with increases in the dose of PAHs injected. Hatchability rat were significantly ($p < 0.05$) decreased with dose exposure increase table (1).

This decline in the hatch abilities was due to embryonic mortalities and teratogenic effects of PAHs which caused default in the hatching processes in fully developed chick embryos. The results are in agreement with [13] who observed that there are physiological abnormality development risks in the chicken embryo and hatched chicks that could be exposed to Ochratoxin A *in vitro*.

Table (1): Show hatchability% and Mortality% of test and control group

Groups	Total no. of eggs	No. of hatched eggs	Hatchability%	Mortality %
Con.	20	18	90	0
A	20	10	50	5
B	20	8	40	10

Also Albers (**2006**) [1] observed that exposure to PAHs in early life of birds cause's developmental abnormalities, decline in hatching success, and biochemical disturbance. Mortalities in the chicken embryos of all groups of this study showed dose-dependent relationship.

Effect of PAHs on liver function:

The results of control and treated chick embryo liver and kidney biochemical studies were presented in the fig 1and 2. In these results there is a significant increase ($p < 0.05$) in the level of liver enzyme (AST, ALT) and decrease in ALP levels with increasing of exposure dose as shown in fig 1.

The results are in agreement with [14] who mentioned considerable changes in chick embryo compared to the control ($p<0.01$) in some biochemical parameters such as ALT,AST, ALP, and amylase after treatment with MA (meglumine antimonite) .

In the present study, increased serum AST, (47.67±2.8,71.7±3.6,43.13±1.16) iu/l, ALT (9.75±2.4,19.50±4.6,7.33±1.8)iu/l concentration and decrease in ALP (12.1±03,10.0±0.2,14.9±1.1) iu/l levels in the chicks' embryo groups(A,B,and Con.) respectively clarify or explain liver damage [15].

The determination of serum enzymes was a useful quantitative indicator for estimating the extent and kind of liver damage in chicken as well as, many other species that were given toxic substances through feed[16], and air [17].

When liver cell plasma is injured, a variety of enzymes is released into the circulation, that cause increasing in the levels of enzyme in the serum [18].

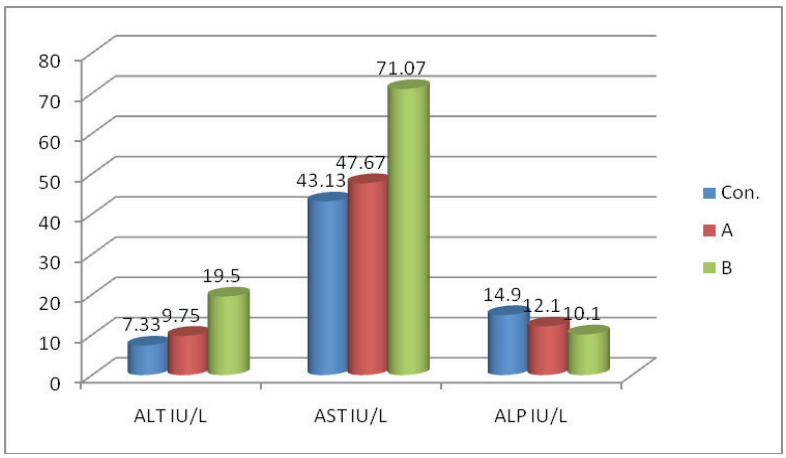

Fig (1): Show the level of liver enzymes (ALT, AST, and ALP) in the serum of chick embryos for groups A, B, and control.

The increase in ALT and AST generated more energy when stressful conditions increase, as a result of increased demand and precursors of carbohydrates to maintain the glycolytic pathway and tolerance in stable levels in the Krebs cycle (Tri Carboxylic Acid) [19].

ALT is found in the cells of various avian tissues and its level varies due to tissue damage. AST is useful for detecting hepatocellular malfunction or disorder and elevation is frequently associated with liver injury.

ALP is an enzyme found in some avian tissues(bone, kidneys, intestine and liver).The increased ALP activity was associated with the Hepatocytes performance due to industrialization increased with an increased biliary pressure , which lead to a blockage of the bile duct due to increase in the concentration of ALP [11].

Changes have been observed in the activity of ALP in tissues, organs and blood of fish when exposed to poisons [20]ALP is playing an important role for energy production during crips cycle (TCA), its help in transport of metabolites across the cell membrane and carboxylic acid cycle [20]ALP is considered as biomarkers for cell toxicity and genetic toxicity [21].

Significant reduction in ALP level in this study is agreement with other researcher which mention that after injection with Benz (k) fluoranthene the alteration in ALP enzyme activity and PCV values was apparent is indicative of the high sensitivity of chick fetuses [22]This result is consistent with studies dealing with the effect of chemical contamination , as the amount of this enzyme decreases compared to the control group .This increase can be adopted as evidence of chemical contamination after chronic exposure.

Effect of PAHs on renal function:

Kidney function in avian species is depended on concentration of urea, uric acid, and creatinine [23]. These three biochemical components and their concentration in peripheral blood have been shown reliable indicators for nitrogen metabolism and renal function [24].

Creatinine and urea values of groups A and B, show significant ($p < 0.05$) differences in their sera concentration (0.41±002mg/dl, 0.61±0.01mg/d for creatinine, 10.66±0.3 mg/d,14.04±0.5mg/dl for urea respectively compared with the values obtained from the chicks embryo belonging to control group (0.27±0.02mg/d for creatinine, 7.06±0.2 mg/dl for urea) as shown in (fig 2).

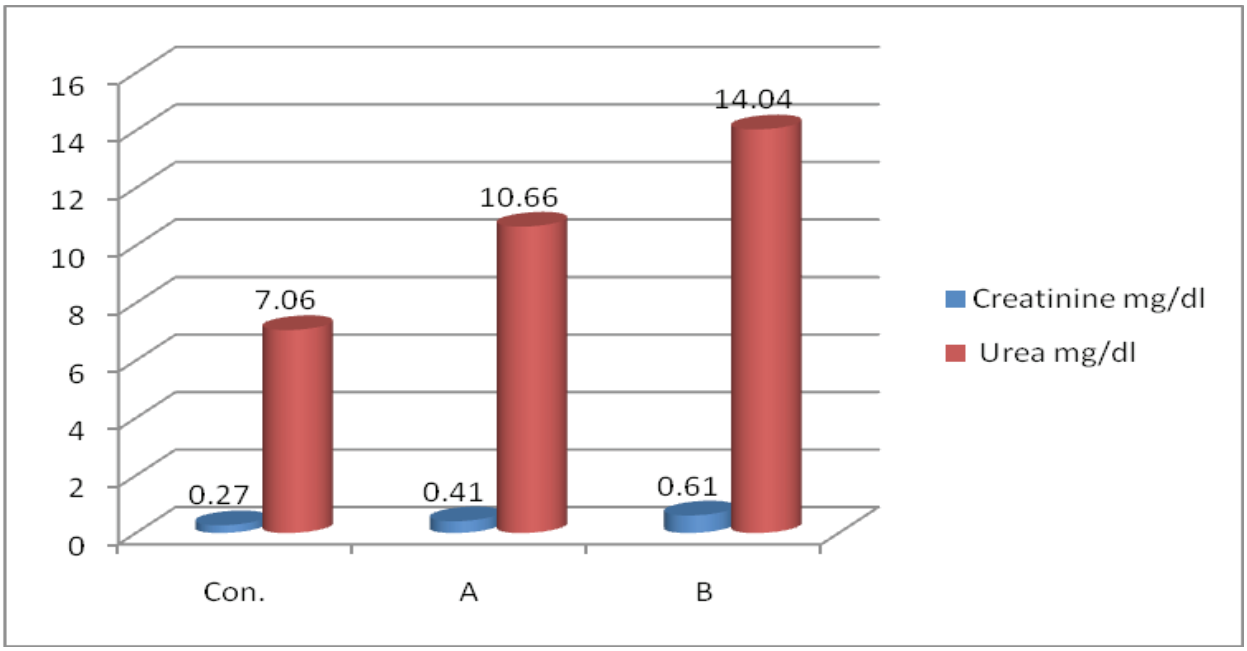

Fig (2): Show the level of Ceratinine and Urea in the Kidney of chick embryos for groups A, B, and control.

These results considered as pathological indicators confirmed the toxicity of PAHs with respect to liver function. Nephrotoxicity also observed by [25].who confirmed that significant increase in ceratinine and serum uric acid levels are indicative of nephrotoxicity in broiler chicken. Nephrotoxic and Hepatotoxic activity of PAHs in different animal species, as indicated by altered serum biochemical profile, is well established.

In the present study, increased serum ALT,AST,concentration and decrease in ALP levels are indicative of liver damage. In the same way, increased urea and ceratinine levels may be due to nephrotoxic effects of *in vivo* inoculation of PAH.

Conclusions

Results showed that exposure to PAHs increased the intensity of these changes such as increased in the level of hepatic enzyme (ALT, AST) and decrease in ALP which means that there is an effect on liver function, as well as there are an rise in ceratinine and urea level which effect on kidney function. That shows an indication of the inefficient treatment of refinery inputs of hydrocarbons into the river before being discharged.

These findings also suggested that there are teratogenic and toxicological risks in the developing chicken embryos that could be exposed to PAHs in *vivo*.

This study shows that the chickens' embryo is a suitable organism to perform early life stage tests. The high sensitivity of chickens' embryo to PAHs supports their use for ecological risk estimations in areas around the river under the influence of refinery activities or other inputs of hydrocarbons into the river.

Ethical Clearance: The Research Ethical Committee at scientific research by ethical approval of Ministry of science and technology in Iraq

Conflict of Interest: The authors have read the manuscript and declare that the work is ready for submission and there is no conflict of interest.

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