Evaluation the exposure of Formalin as a Disinfectant For Poultry House on Hematological, Biochemical Parameters and Histopathological Examination in Broiler

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Abstract

This study was done to evaluate exposure to inhalation of formalin as a disinfectant for poultry house on hematological, biochemical parameters and histopathological examination in broiler. The used (24) chicken divided into two groups, every group included (12) chicken. First group (control) and second group exposure to formalin by inhalation for 2 weeks. Blood samples were collected at end of each week for analyzed some hematological such as (PCV, Hb, RBCs, total WBCs counts and differential WBCs count) and biochemical parameters such as (total protein, albumin, globulin and bilirubine) at the end of each week after 1wk scarified of half number of broiler and remove organs such as (lung, heart and liver) for histopathological examination and end of 2wk scarified the reminder number of broiler.In our study the hematological changes revealed significant decreases into PCV, Hb, RBCs and WBCs counts after 1wk and 2wk compared with control group. The total protein, albumin, and globulin showed significant decreases while bilirubine showing significant increasing after 1wk and 2wk of treated group compared with control group. The differential WBCs count showed significant increasing into neutrophils, and monocytes after 1wk while there was significant increasing into eosinophils, basophils, and neutrophils after 2wk compared with control group. There was significant decreasing into lymphocytes eosinophils and basophiles after 1wk of treated groups, but after 2wk the monocytes and lymphocytes that showed significant decreasing. The histopathological examination of lung in treated group after 1wk revealed highly changes that represent by congestion of blood vessels, hemorrhages with thickening of blood vessels walls, thickening into alveolar walls, alveoli filled with erythrocytes and fibrin, hemosidrosis, and hyperplasia of alveolar cells into treated group compared with control group, while after 2wk the changes was emphysema (destruction of alveolar walls) only. The heart revealed congestion of blood vessels after 2weeks. The liver revealed more changes that represented by congestion of central veins and necrosis in the hepatocytes, fibrosis and hyperplasia into portal area of the liver tissues compared with control group. Changes into liver after 2wk of treated group showed acute inflammation, congestion of blood vessels, hemorrhage and degeneration of hepatocytes with necrosis into some areas. After 1wk the heart show slightly changes includes congestion of blood vessels and slightly infiltration of inflammatory cells and hemorrhage compared with control group, but after 2wk the heart show more infiltration of inflammatory cells with fibrosis of treated group compared with control group.

Key Word: Formalin, Hematological and Biochemical Parameters, Histopathological Examination, Broiler.

Introduction

Formaldehyde (FA) (HCHO : IUPAC name : metanal) [1,2,3 and 4]. FA is highly soluble in water ,as well as in most organic solvent, and is highly reactive molecule that can be irritating to tissues through direct contact [5, 6 and 7].Formaldehyde (HCHO) is a colorless, flammable gas with a pungent, suffocating odour. It is soluble in water, acetone, benzene, diethyl ether, chloroform and ethanol. HCHO used to make plastics and resins for the production for of intermediates and other miscellaneous uses. HCHO also used as disinfectant in many human medicines and cosmetics, as an antiseptic in veterinary drugs and biological and in fungicides. textiles and embalming fluids [21] (IARC, 1982). Exogenous HCHO is taken up into human body by ingestion, inhalation and dermal exposure. Inhaled HCHO appears to be readily absorbed by the upper respiratory tract but is not distributed throughout the body because of its rapid metabolism [22,23](Casanova et al., 1988; Heck et al., 1985). FormalineTM (Vétoquinol) is a general disinfectant and can be used as a fumigant to decontaminate all surfaces of a building, if the building can be tightly sealed. Adding potassium permanganate to the FormalineTM solution causes a reaction that releases formaldehyde gas. Profilm® is a time-release formaldehyde fumigant for poultry houses. Formaldehyde has a broad spectrum of activity and is highly effective as it kills most bacteria and fungi; however it must be used carefully due to the irritating fumes and potential explosiveness. Formaldehyde gas is one of the few agents

1. Animals :- In our study we used (24) chicken divided into two groups, every group included (12) chicken. First group as control and second group was exposured to formalin by inhalation and the specimens of blood and tissues were taken after 1wk and 2 wk. These animals maintained in air conditioned quarters $(24C\square)$ stander husbandry condition, animals were given prepared ration.

2. Blood Sampling:-

Blood was collected from wing of each chicken; 2 ml of each was dispensed into clean container containing anticoagulant EDTA. The rest was allowed to clot. The anticoagulant blood was used to determine red blood cell (RBC) count, white blood cell (WBC) with haemocytometer, packed cell volume (PCV) was determined using Vol./12 No./22013

effective against coccidiosis and cryptosporidiosis. The facility must be completely depopulated and the building must be sealed for 24 hours. Do not enter until it has been thoroughly ventilated. Formaldehyde is classified as a probable carcinogen the human by U.S. Environmental Protection Agency. Formaldehyde can cause allergies, and is part of the standard patch test series [8]. However, FA is highly irritant to mucous membrane [9]. Kilpstein and Metz, allowed two rabbits inhale formaldehyde gas. The first of these inhaled gas for one-half h.; the others for one-half h on three consecutive days. The post-mortem examination showed rhinitis, laryngitis, trachietis, and bronchitis [10].Histological catarrhal examination revealed distinct inflammation changes in the lung of the animals. Many of the alveoli are filled with homogeneous redstaining exudates. Many PMNLs are found in the capillaries, in the connective tissue of and lung free in the alveoli. The eosinophiles are numerous and mononuclear leukocytes are present but few in numbers [11].

Material and Method

microhaematocrit method, while the haemoglobin concentration was determined by Sahel. Blood smears were stained with Giemsa stain for differential white blood cells count[12].

3. Blood Parameters:-

Blood was collected through the heart from each chicken; 2 ml of each was dispensed into clean container without anticoagulant to obtained serum. The parameters that examined were total protein g /L, albumin g/L, globulin g/L, bilirubine mg/L [13].

4. Histopathological Examination: -

The samples obtained from the following organs (lung, liver, and heart). These tissues fixed with formalin then dehydrated by graded alcohol, this is followed bv dealcoholization with xylol and embedding with paraffin wax and blocking. Histological sections of 5-6 μ thickness were obtained by a microtom. Dewaxed , dehydrated, and staining by hematoxlin and eosin stain, from all chickens of all organs [14].

5.Statistical Analysis

1- The data were statistically analysed using analysis of variance(ANOVA). The

computerized SPSS (Statistical Packages for the Social Sciences) (V.13) program were used.

2-The data were expressed as mean \pm standard deviation (mean \pm SD). Least significant different test (LSD) was used to test the difference between means (groups); $p \le 0.05$ was considered significant [24].

Results

• Hematological Examination:-

1.1Effect of formalin on blood Parameters after 1wk and 2wk in Broiler. In the Table [1] showed that changes into PCV, Hb and WBCs are significant decreases (P < 0.05) in group treated with formalin compared with control group after 1wk and 2wk.

Blood	PCV		Hb		RBCs $\times 10$		WBCs \times 10	
	g/L		g/L					
Parameters	Control	Formalin	Control	Formalin	Control	Formalin	Control	Formalin
Periods								
(1wk)	41.5 ±	27.8 ±	11.2 ±	8 ±0.91*	$5.63 \pm$	3.92 ±	6.10 ±	$3.86 \pm$
(N=12)	0.3	1.12 *	0.52		0.47	0.85 *	1.9	0.31 *
(2wk)	40.98	25.06 ±	$11.56 \pm$	7.4±0.52*	$5.84 \pm$	3.72 ±	$6.28 \pm$	$4.67 \pm$
(N=6)	±3.46	2.40*	1.39		0.73	0.69*	0.57	0.87 *

N= number of animal, Mean ± SD,* P< 0.05

1.2 Effect of Formalin on Differential White Blood Cells count after 1wk and 2wk in Broiler.

Table [2] showed the type of leukocytes increasing into blood picture or differential count of WBCs, the eosinophils and monocytes showed a lot increasing into number compared with control group but the all types of cells significantly increasing after 1wk. While there are significant increasing in the percentage of neutrophil, basophiles and eosinophil. while there is decreasing into percentage of monocyte and lymphocyte percentage.

Periods	(1wk)) (N=12)	(2wk)(N=6)		
Types of WBCs	Control	Formalin	Control	Formalin	
Neutrophil	43.91 ± 0.11 %	50.92 ± 0.13 % *	44.07 ± 3.62 %	58.81 ± 6.40 %*	
Basophil	2.6 ± 1.45 %	4.72 ± 0.43 % *	0.42 ± 0.01 %	6.29 ± 0.84 % *	
Eosinophil	5.4 ± 0.12 %	10.81 ± 0.76 % *	2.47 ± 0.04 %	7.91 ± 0.84 %*	
Monocyte	8.01 ± 0.35 %	15.74 ± 0.32 % *	10.36 ± 0.72 %	30 ± 0.53 %*	
Lymphocyte	42.04 ± 0.78 %	21.82 ± 0.67 % *	46.32 ± 3.16 %	19.04 ±0.31 %*	

Table [2] Effect of Formalin on Differential White Blood Cells count after 1wk and 2wk in Broiler

N=number of animals, Mean ±SD, *=P< 0.05

2.Biochemical Analysis:-

2.1Effect of Formalin on Biochemical parameters after 1wk and 2wk in Broiler.

Table [3] showed significant decreasing into total protein, albumin and globulin (g/L) percentage while there is significant decreasing percentage to bilirubine mg/L after1wk compared with control group. There are significant decreasing in the percentage of total protein, albumin and globulin percentage after 2wk of control group. There are significant increasing into bilirubin percentage after 2wk compared with control group.

Table [3] Effect of Formalin on Biochemical parameters after 1wk and 2wk in Broiler.

Blood parameter				
Groups	Total protein	Albumin	Globulin	Bilirubin
-	g/L	g/ L	g/L	mg/L
Control (1wk)(N=12)	7.10 ± 0.41	4.38 ± 0.78	2.71 ± 0.51	1.72±0.036
Formalin (1wk)(N=12)	± 0.23*	2.37±0.18*	$1.46 \pm 0.63*$	2.94±0.012*
Control (2wk) (N=6)	7.02 ± 0.65	4.51 ± 0.29	2.49±0.23	1.67±0.01
Formalin (2wk)(N=6)	$3.62 \pm 0.14*$	2.42±0.39*	1.20± 0.48*	4.96±0.29*

N=number of animals, Mean \pm SD, *= P< 0.05

Histopathological Examination:-

1. Lung:-

The lung show highly changes after 1wk that represent by congestion of blood vessels and hemorrhages with thickening of blood vessels walls, and thickening into alveolar walls, alveoli filled with erythrocytes and fibrin, hemosidrosis, and hyperplasia of alveolar cells into treated group compared with control group, while after 2wk the changes was emphysema (destruction of alveolar walls) only.

2.Heart:-

The histopathological examination of heart after 1wk into treated group revealed changes includes congestion of slightly blood vessels and slightly infiltration of inflammatory cells hemorrhage and compared with control group, but after 2wk more infiltration of the heart show

inflammatory cells with fibrosis of treated group compared with control group. 3.Liver:-

The liver revealed more changes that represented by congestion of central veins and necrosis into the hepatocytes, fibrosis and hyperplasia into portal area of the liver

The formaldehyde is accepted that toxic carcinogenic and slightly over certain concentrations, and the harmful effects of formaldehyde (FA) increase under room temperature conditions, because the molecule easily evaporates [15,1,6,16, 2] and 3].In our study of [17] that revealed significant decreasing into blood picture after 1wk and 2wk from exposed to formaldehyde. This result attributed to formaldehyde lead to anemia. The result of differential WBCs counts attributed to inflammation in internal organs. This result histopathological indicated by results to studied organs. The result of changes biochemical changes attributed to also effect of formaldehyde on histopathological in liver. In our study changes the histopathological changes the lungs in revealed congestion hemorrhage and thickening into blood vessels and alveolar wall, the alveoli filled with fibrin. Another study into the formalin and formaldehyde revealed distinct inflammatory changes into the lungs of rats and ginea pigs show marked congestion, many of alveoli filled with a homogenous red-staining exudates, polymorph nuclear leukocytes are found in the capillaries, in the connective tissue of the lung and free in the alveoli. Another study the female rabbits that exposed to toluene found thickening into alveolar infiltration and proliferation of walls. inflammatory dilatation of cells, bronchioles emphysema, fibrin into the alveolies and hemorrhage [18]. Another study on the guinea pigs that exposed to TDI (Toluene Di Isocynate) showed the histopathological changes interstitial inflammation. thickening pleural and

tissues compared with control group. Changes into liver after 2wk of treated group showed acute inflammation. congestion of blood vessels, hemorrhage and degeneration of hepatocytes with necrosis into some areas.

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Discussion

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peripheral lymphoid hyperplasia [19]. The histopathological changes in the liver into our study consistent with another study on rabbits that found degeneration, necrosis, congestion into blood vessels. and infiltration of inflammatory cells [11]. General changes produced by formalin in the liver, these consist essentially of cloudy swelling, vary in intensity associated with vacuolation of protoplasm and destructions of nuclei and total destruction of cell may occurs. The changes into the heart of chickens in our study consistent with [20] that obtain our result from the study on rabbits at very high level exposure -such as might occur in an enclosed space or during a spill-toluence can injure the liver and kidney, necrosis or fatty degeneration of heart. liver. and adrenal.Epidemiological findings suggesting that formaldehyde exposure is associated with a higher risk on general health of animals and human and effect on hematological changes. It have led to consideration of the potential mechanism of action by which inhalation of this rapidly reactive agent can cause changes in bone marrow. Some research investigation the formaldehyde consideration as а leukemogen have been the difficulty in envisioning how inhaled formaldehyde might penetrate to the bone marrow; and the lack of similarity of non-cancer effects to other known human myeloleukemogens. particularly the absence of pancytopenia in humans or laboratory animals exposed to high levels. However, both of these arguments have been addressed by the recent finding of a pancytopenic effect and abnormalities chromosomal in heavily exposed Chinese workers which. if

replicated, are indicative of a genotoxic effect of formaldehyde on hematopoietic stem cells that is in keeping with other known human leukemogens. Review of the body of evidence suggests an apparent discrepancy between studies in laboratory animals, which generally fail to show evidence of penetration of formaldehyde into the blood or evidence of blood or bone marrow genotoxicity, and studies of

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exposed humans in which there tends to be evidence of genotoxicity in circulating blood cells. One possible explanation for this discrepancy is species difference. It can be concluded from the present study that formaldehyde has no useful effects; rather, at higher levels it has adverse effects on health and performance of broilers hen given for a prolonged period of time.

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أجريت هذه الدراسة لتقييم التعرض للفور مالين كمعقم لبيوت الدواجن عن طريق الاستنشاق على المعابير الدمية والكيموحيوية والفحص الامراضية النسجية لدجاج اللحم استخدمت في هذه التجربة 24 دجاجة قسمت إلى مجموعتين كل مجموعة تتضمن 12دجاجة المجموعة الأولى تعتبر سيطرة والمجموعة الثانية تتعامل مع الفورمالين عن طريق الاستنشاق لمدة أسبوعين .تجمع عينات الدم نهاية كل أسبوع وبعض ,differential WBCs count, total WBCs counts) RBCs counts, Hb, PCVتحليل بعض المعابير الدمية مثل)المعايير الكيموحيوية مثل)البروتين الكلي الألبومين الكلوببولين البليروبين (وبعد الأسبوع الأول تذبح نصف عدد الحيوانات لإزالة الأعضاء الداخلية مثل)الرئة و القَّلب والكبد (لفحص الامراضية النسجية.وفي نهاية الأسبوع الثاني يتم ذبح العدد المتبقى من دجاج اللحم .وأظهرت نتائج الدراسة الحالية حصول ا انخفاض معنوي في و كذلك حصول انخفاض معنوي في البروتين الكلي والألبومين و (total WBCs) counts, total RBCs count, Hb, PCV الكلوبيولين في كلا الأسبوعين بينما حصل زيادة في مستوى البليروبين وأظهرت دراسة العد التفريقي لكريات الدم البيضاء زيادة في كريات العدلة وكريات وحيدة النواة في الأسبوع الأول إما كريات الدم البيضاء الحمضة والقعدة وكذلك كريات الدم اللمفاوية فقد حصل فيها نقصان معنوي بعد الأسبوع الأول بينما في الأسبوع الثاني فقد حصل زيادة في كريات الدم البيضاء العدلة والحمضة والقعدة ونقصان في كريات الدم اللمفاوية ووحيدة النواة .وقد أظهرت نتائج فحص الامراضية النسجية للأعضاء المدروسة حصول تغيرات نسيجية في الأعضاء المدروسة آذ لوحظ احتقان الأوعية الدموية ونزف مع تثخن جدار الوعاء الدموي بعد الأسبوع الأول كذلك تثخن جدار الاسناخ وامتلاؤها بكريات الدم الحمراء واللفين والهيموسدرين وزيادة في التنسج بينما بعد الأسبوع الثاني نلاحظ تحطم في جدران خلايا السنخية أما القلب فقد لوحظ احتقان الأوعية الدموية بعد كلا الأسبوعين بينما الكبد فقد لوحظ فيه احتقان الوريد المركزي والتنخر خلايا الكبدية وتليف وزيادة التنسج في المنطقة البابية مقارنة مع السيطرة إما في الأسبوع الثاني فقد لوحظ التهاب حاد في وتنخر وكذلك احتقان الأوعية الدموية ونزف وإنحلال في خلايا الكبدية