

## Nephropathy Effects of 2,3,7,8-Tetrachlorodibenzo-P-Dioxin (TCDD) Toxicity In Ova Injected Chicken

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### Abstract

Eggs are importance in human feeding, it is inexpensive and complex source of food with highly biological value, The aims of this study to investigate the evaluation toxicity of pollution of biological tissue characterized by 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) toxicity in kidney chickens. Chicken eggs are attractive bioindicator of pollution. Sixty chicken eggs fertile supplier from local market of Baghdad province from Alzafarany city, these eggs were divided equally and randomly into three equal groups: 1<sup>st</sup> group injected into air cell with 0.2 µg/ kg corn oil at day 4, 2<sup>nd</sup> group injected in air cell at day 4 with 0.1 µg/ kg TCDD dissolve in corn oil, 3<sup>rd</sup> group injected in air cell at day 4 with 0.2 µg/ kg TCDD dissolve in corn oil. The eggs incubated for 21 days. Biochemical assay (Creatinine and Urea) concentration and pathological examination were observed on chicken at days 15 and 30. Biochemical assay of kidney function found significant increased  $P < 0.05$  in serum urea concentration & creatinine concentration at days 15 & 30, with highly increased in dose 0.2 µg/ kg at day 30. Pathological lesion characterized by chest and abdominal edema, hydronephrosis with severe tubular and glomeruli damage, in addition of destruction with extensive hemorrhage & lymphocytic infiltration.

**Keyword:** 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD), ova chicken, nephropathy, urea & creatinine.

### INTRODUCTION

Chicken eggs are assumed to be professional bio-indicators of environmental pollution like dioxins contamination and for human health risk, so the low intake may cause highly levels in eggs ( Anastasia *et al.*, 2020 ), chicken eggs like liver and adipose tissue have congener profile ( Chang *et al.*, 1989 ). Eggs are a highly nutritious food vital vitamins, rich minerals, saturated and unsaturated fatty acid which play a significant role in maintaining health ( Molnar & Osi, 2020 ).

The common term dioxin is often used for a number of lipophilic chemical compounds (polychlorinated dibenzofurans- PCDFs), (poly chlorinated dibenzo- para dioxins- PCDDs) including certain dioxin-like polychlorinated biphenyls- PCBPs with similar toxic properties, whoever the denomination of dioxin is given to 2,3,7,8- tetrachloro- dibenzo- para-dioxin (TCDD) and presence as environmental pollution ( Stantaon *et al.*, 2001 ), present in herbicides contamination, paper bleaching, volcanic activities, cigarette smoke, forest fire etc.

Human are exposed to dioxins via food contaminatin mostly fish, diary products, milk and eggs.( BMU, 2013 & UNEP, 2013)

Eggs from laying hen's housed outdoors are sensitive indicator to dioxin contamination in soil, also be a relevant pathway in human ( Digangi & Petrlik, 2005; Weber *et al.*, 2015 ). TCDD exposure caused adverse health outcome mostly hypothyroidism, infertility, birthdefect, neurobehavioural disorder, immune system deficiency, nephropathy and kidney cancer.( Smith & Loper, 2001; Baan *et al.*, 2009 & Mannetje, 2012 ).

## **MATERIALS AND METHODS**

### **Experimental eggs and chickens:**

In Baghdad province in Iraq from Alzaafarana city near Diyala river where is highly percent of pollution, 60 fertile eggs from local market were supplier the fertile eggs were artificially incubated at 37C<sup>o</sup> and 55% relative humidity in incubator (model 21) humidaire for 21 day.

### **Chemicals:**

Stoek solution P> 99% of 2,3,7,8- tetrachloro-dibenzo-para-dioxin(TCDD) were supplier from Sigma chemical company (St. Louis,Mo.) and dissolved freshly in corn- oil.

### **Eggs injection procedure:**

Sixty (60) fertile chicken eggs (ova) were divided randomaly and equally into three group: 1<sup>st</sup> group (C) control group contain 20 ova ingected 0.2µg/ kg corn oil in the aircell at day 4 of incubation ( Sanderson & Bellward, 1995 ), 2<sup>nd</sup> group contain 20 fertile eggs were injected 0.1µg /kg TCDD after dissolved freshly in corn oil in the aircell at day 4 ,while 3<sup>rd</sup> group contain 20 fertile eggs injected 0.2 µg /kg TCDD in the aircell after dissolved freshly in corn oil.

These perinatal time were chosen to recorded the pathological lesion which may accur due to TCDD toxicity during embryogenesis of fetus in the kidney ( Scanes *et al.*, 1987 ). Afrt 21 days of incubator chicken were housed in hatcheng trays on the floor of incubator of the same humidity and temparture used for fertile eggs. The experiment were located in the animal house resources/ Science and Technology which located in Al- Zaafarana city, Baghdad, Iraq.

After 21 days the hatched chicken were euthanized with Co<sub>2</sub> for 45 sec. for all groups in average 10 chicks at days 15 and 10 chicks at days 30 of the experiment.

### **Blood collection:**

Blood were colleded from newly hatched chicken at days 15 and 30 from all groups by cardiac puncture after chest cavity opening by euthanized with CO<sub>2</sub> for 45 sec., then the blood centrifuged at 3000g, 4C<sup>o</sup> for 20 min., under -20C<sup>o</sup> plasma was stored.

### **Biochemical assay:**

#### **a) Blood urea concentration assay (mL/dL):**

Enzymatic and colorimetric method based on the specific action of urea which hydrolyse urea in ammonium ion carbon dioxide complex, this coloration proportional to urea concentration and measured at 600nm.( Fawcett & Scott, 1960 )

#### **b) Serum creatinine concentration assay (mg/dL):**

Creatinine under alkaline condition react with picrate ions forming reddish complex. The formation rate of the complex is measured through the increases of absorbance in apre-fixed interval of time is proportional to the concentration of creatinine in the sample.

Creatinine + picric acid  $\frac{PH>12}{37C}$  red addition complex.

Calculation test (A1+A2) / Standard (A1+A2).

Standard concentration: 2 mg/dl. ( Henry *et al.*, 1974 )

### Pathological examination:

After blood collocation as above, the abdominal cavity were carfully cut, kidneys were carefully excised for detect any abnormal lesion, size, color, density and adhesion, etc. then and according to ( Bancroft & Gamble, 2008 ), kidney samples  $>1\text{cm}^3$  were immersed in Bouin's solution and embedding in paraffin section, then stained by hematexylin & eosin stain and examined by light microscope.

### Statistical analysis:

All the grouped data were statistically read by SPSS program, Version 17 software (2010). Testing methods including one way ANOVA for comparisons among groups. P values of less than  $<0.05$  were considered statistical significance. All data were expressed as means  $\pm$  standard error (SE) ( Leech *et al.*, 2011; Bassim *et al.*, 2020 and Humadi *et al.*, 2021).

## RESULTS

### Biochemical assay:

The table ( 1 ) showed significant increase (  $P<0.05$  ) in serum urea concentration (ml/dL) in 3<sup>rd</sup> compared with control group.

Table (1):Chicken serum urea concentration (ml/dL) in study groups.

Groups	15 days	30 days
Control (1 <sup>st</sup> group)	9.6 b	9.1b
TCDD 0.1 $\mu\text{g/kg}$ (2 <sup>nd</sup> group)	13.2 ab	13.7 ab
TCDD 0.2 $\mu\text{g/kg}$ (3 <sup>rd</sup> group)	19.2 a	19.4 a

n:10 with  $P<0.05$ .

Also the table ( 2 ) showed significant increase (  $P<0.05$  ) in serum creatinine concentration (mg/dL) in 3<sup>rd</sup> group compared with control group.

Table(2): Chicken serum creatinine concentration (mg/dL) in study groups.

Groups	15 days	30 days
Control (1 <sup>st</sup> group)	0.96 b	0.97 b
TCDD 0.1 $\mu\text{g/kg}$ (2 <sup>nd</sup> group)	1.95 ab	1.98 ab
TCDD 0.2 $\mu\text{g/kg}$ (3 <sup>rd</sup> group)	2.7 a	3.1 a

n:10 with  $P<0.05$ .

### Pathological examination:

At day 15 and 30 no important pathological changes were observed in chicken kidney control (1<sup>st</sup> group), while severe abdominal and chest sac edema covered the internal organ showed in both 2<sup>nd</sup> and 3<sup>rd</sup> groups (0.1 and 0.2)  $\mu\text{g/kg}$  TCDD respectively at (15 and 30) day with sever hydronephrosis. At 15 days, kidneys of chicken in both treated groups showed dilation of proximal convoluted tubules epithelium lining , some area swelling and granular

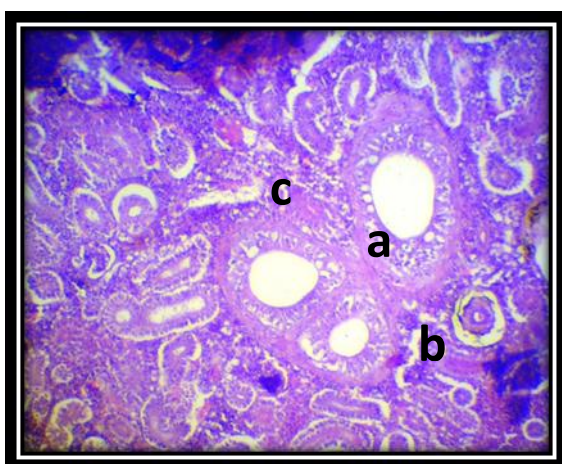
(severe acute cellular swelling), other are sloughing and desquamated cells in lumen with atrophied tubules, moderated mononuclear cells monocytes infiltration. fig(1 and 2).

Other section of kidney consist atrophy of proximal convoluted tubules with sever interstitial lymphocytes infiltration and densety amorphous eosinophilic (colloid) cast in tubules. fig(3).

In newly hatched chicken treated with (0.1 $\mu$ g /kg TCDD) kidney at days 30, recorded absences of most glomeruli capsule, distended and width congested blood vesels, colonized most of tubular epithelial cells fig(4). Extensive hemorrhage contain the cortex area causing tubular and glomerular atrophy with interstitial edema. fig(5). Follicular like hemorrhagic area causing atrophy in tubules and glomeruli with amyloid like material. fig(6). Balloning distended blood vesels, congested with blood with multiple tubular colloid hyaline cast degeneration and sever lmphocytic cuffing around blood vesels with cells damage and necrosis in cortical layer fig(7).

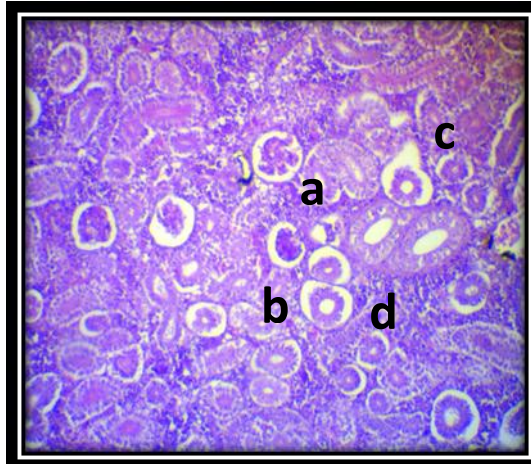
At days 30, kidneys of the hatched chicken treated with 0.1  $\mu$ g/kg TCDD group showed necrotic all of glomeruli and tubules , all tubules degenerated with stare-shape like (hydropic degeneration) due to enlargement and swelling of tubular epithelial cells in lumen with colonized epithelial cells and glomeruli atrophy. fig(8). Extensive interstitial of hemorrhage causing damage and destrution of cortex layer. fig(9,10,11).

Group of 0.2  $\mu$ g /kg TCDD at day 30 observed sever damage and necrosis of tubules with different degree of tubules colloid cast and congested blood vesels. Fig(12,13,14), Kidney at fig(15 and 16) showed severe damage due to extensive hemorrhage & interstitial infiltration of mononuclear calls causing cortex atrophy.

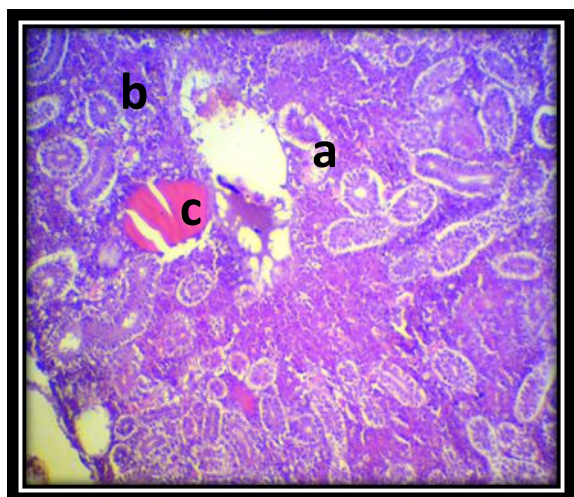


**Figure (1):** Kidney of newly hatched chicken treated by 0.1  $\mu$ g /kg TCDD at days 15 showed:a) dilation of proximal conulted tubules epithelium lining b) sloughing & desquamation of epithelial cell in lumen c) atrophied tubules.

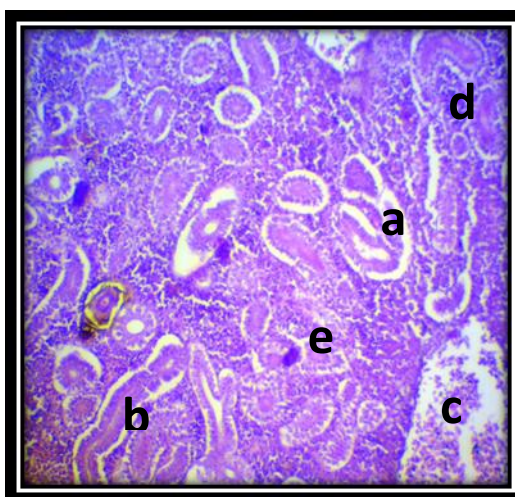
( X20 H&E )



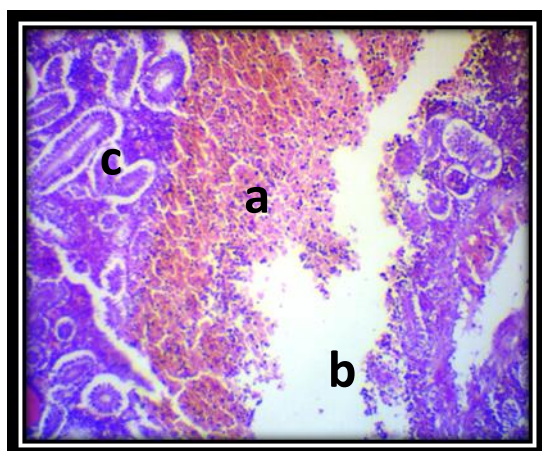
**Figure (2):** Kidney of newly hatched chicken treated by 0.1  $\mu$ g /kg TCDD at days 15 showed:a) dilation of proximal conulted tubules epithelium lining b) sloughing & desquamation of epithelial cell in lumen c) atrophied tubules d) interstitial infiltration of lymphocytes ( X20 H&E ).



**Figure (3):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 15 showed:a) sloughing epithelium lining cell in lumen b) severe lymphocytic infiltration in interstitial layer c) amorphous eosinophilic colloid cast in tubules ( X20 H&E ).

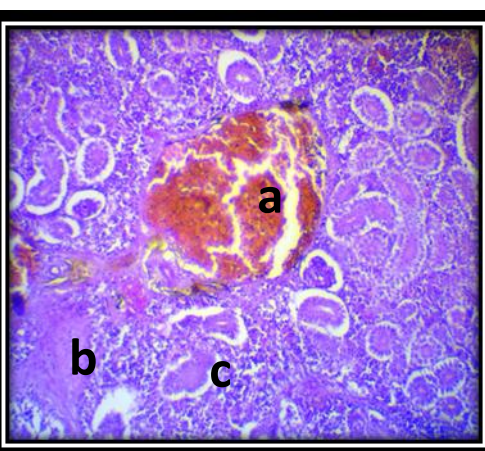


**Figure (4):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed:a) tubular epithelial cells sloughing b) closed irregular proximal convoluted tubules c) distended & wide congested B.V. d) absence of corpora e) colonization of tubules epithelium ( X20 H&E ).



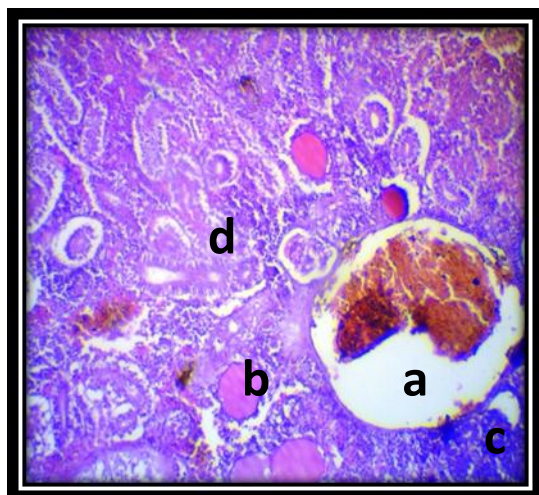
**Figure (5):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed:a) extensive interstitial hemorrhage b) edema c) tubular acute cellular swelling.

( X20 H&E ).



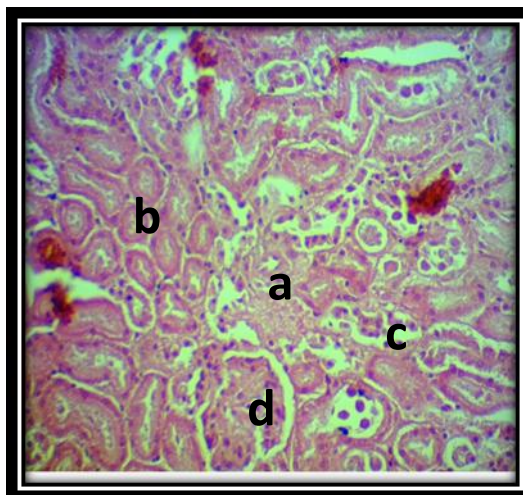
**Figure (6):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed:a) follicular like hemorrhage b) amyloid like materials c) acute cellular swelling.

( X20 H&E )



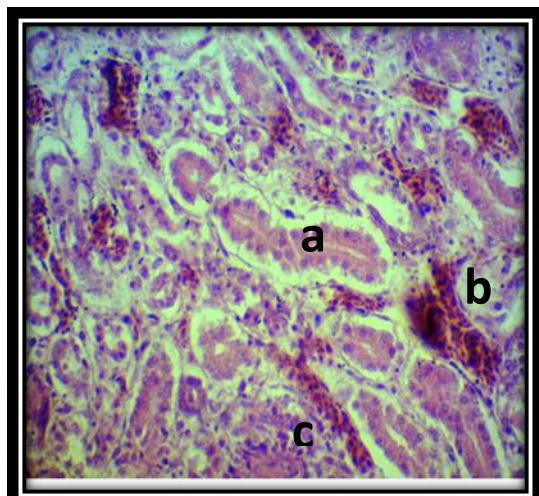
**Figure (7):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed:a) ballooning distended B.V. & congested b) tubular colloid hyaline degeneration cast c) cuffing of lymphocyte around B.V. d) necrosis

( X20 H&E )



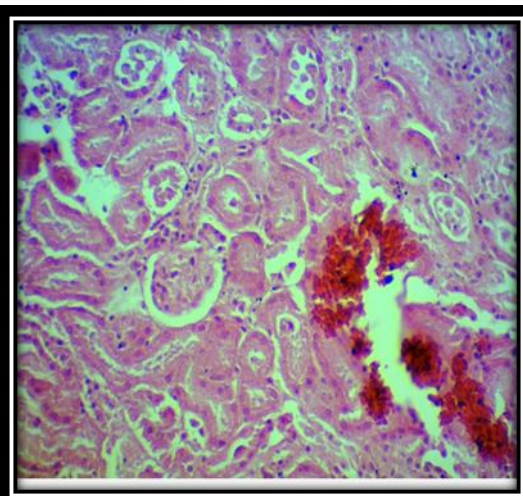
**Figure (8):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed:a) necrosis of glomeruli & tubules b) star shaped degenerated tubules ( hydropic degeneration ) c) colonized of tubular epithelium d) glomeruli atrophy.

( X20 H&E )



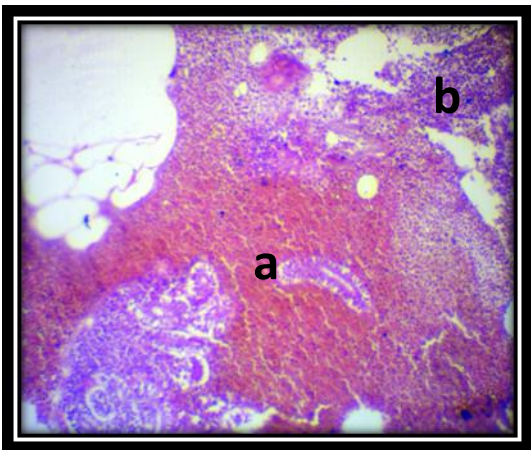
**Figure (9):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed:a) acute cellular swelling of tubules b) severe interstitial hemorrhage c) infiltration of lymphocytic cells.

( X40 H&E )



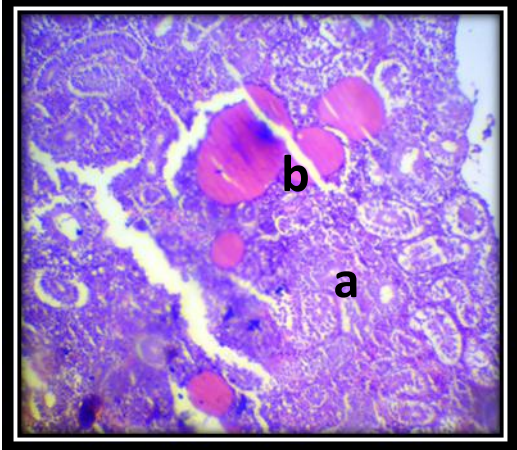
**Figure (10):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed damage of cortical area by extensive hemorrhage with mild lymphocytic infiltration.

( X40 H&E )



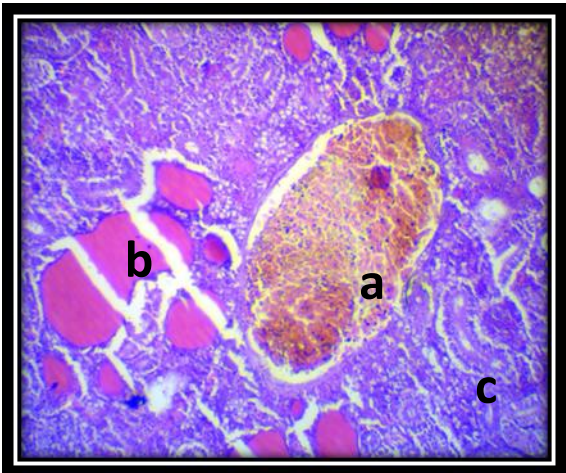
**Figure (11):** Kidney of newly hatched chicken treated by 0.1 µg /kg TCDD at days 30 showed:a) damage of cortical area by extensive hemorrhage with atrophy of glomeruli & tubules b) severe interstitial lymphocytic infiltration.

( X20 H&E )



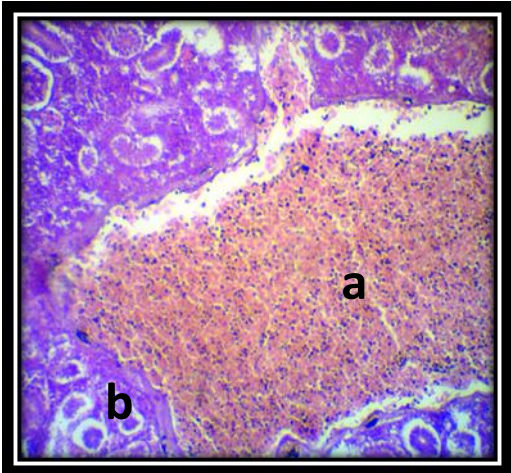
**Figure (12):** Kidney of newly hatched chicken treated by 0.2 µg /kg TCDD at days 30 showed:a) damage of tubules b) colloid tubular cast.

( X20 H&E )



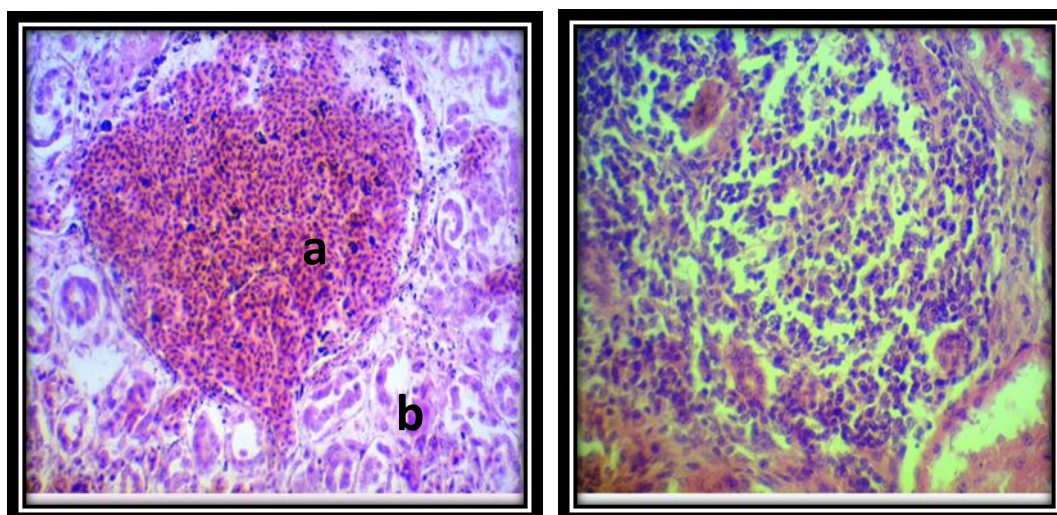
chicken treated by 0.2 µg /kg TCDD at days 30 showed:a) severe dilated congested B.V. b) colloid tubular cast c) mild lymphocytic infiltration.

( X40 H&E )



hatched chicken treated by 0.2 µg /kg TCDD at days 30 showed:a) severe dilated congested B.V. b) swelling of tubules.

( X40 H&E )



**Figure (15):** Kidney of newly hatched chicken treated by 0.2 µg /kg TCDD at days 30 showed:a) severe interstitial hemorrhage surrounded by fibrous connective tissue b) damage & necrosis of tubules.

( X40 H&E )

**Figure (16):** Kidney of newly hatched chicken treated by 0.2 µg /kg TCDD at days 30 showed interstitial infiltration of lymphocytes surrounded by fibrous connective tissue.

( X40 H&E )

## DISCUSSION

Kidney function recorded significant increased in blood urea and creatinine concentration at (0.1 and 0.2) µg/kg TCDD specially at day 30, insufficiency of renal function occur due to distrubance in  $\text{Na}^+\text{K}^+$  pump of nephrone due to epithelial cell detachment from tubules and destruction of renal parenchyma, dioxin enter the body in the food that chicken eat then enter circulatory system to adipose tissne and liver cells ( Birkner *et al.*, 2008 ).

In liver TCDD ties to protein aryl- hydrocarbon (Ah- receptor) then tie to secondary protein. Ah- receptor- nuclear translocation.(Ah- rnt), this protein converts protein- dioxin complex in cell nucleus, in nucleus ties to strand turn on and turn off causing multiple disease like nephropathy, cancer, immunosuppression & hydronephrosis ( Silbergeld & Gasiewicz, 1989; Greenfact, 2012 and Lali, 2018 ).

In human hydronephrosis is found in 1.5-3.3% of all outepsies and 1-5% of pregnancies on antenatal ultrasonography, the major of hydronephrosie is anatomical obstruction of urinary tract from kidney to bladder via the ureter causing increase retrograde hydrostatic pressure causing destruction of kidney paranchyma and hydronephrosis ( Lee *et al.*, 2006; Frokiaer & Zeidel, 2011 and Yamacake & Nguyen, 2013 ), Chick edema which present in the chest and abdominal cavity due to toxicity of TCDD which called mysterious disease ( Hites, 2011).

Pathological change characterized by acute cellular swelling in proximal convoluted tubules with damage of tubules and glomeruli at day 15 with dose ( 0.1 and 0.2 µg/kg ) TCDD and increased the damage at day 30 at dose 0.2µg/kg by sever hemorrhage and massive

lymphocytic infiltration, these results are in agree with ( Humadi, 2019 ) who showed dilated tubules, enlargment of glomruli with glomeruli tuft, tubular proteinaceous material and granuloma after exposed to toxic materials ( Acrylonitrile ).

The study of ( Courtney & Moore, 1971 ) found that TCDD when given in pregnant murins (rat and mice) causes abnormalities in kidney like hydronephrosis in fetus at dose (1-3)µg/kg on GD10 or from GD10- GD13 and reduction in kidney size, dilation of renal calyces, loss of renal papilla which cause ureteric ooclusion & the lumen anatomically obstruct due to hyperplasia induced by TCDD, then leading to renal paranchyma damage and necrosis ( Abbott *et al.*, 1987 and Nishimura *et al.*, 2006 ), So these results agree with ( Humadi *et al.*, 2021 ) who showed pathological changes charaterized by present of multiple granuloma with giant cells and calcification at days 90 indicated that TCDD cause liver damage depending on dose and period of exposure and early indicator to liver cancer special with present of pleomorphism irregular epithelioid granuloma.

TCDD administration measured the antibody immunoreactivity against CYPIA and activation of Ah-r dependend gene on outerzone of the medulla which caus the tissue damage ( Fujiwara *et al.*, 2008 ).

The present of inflammatory cells mostly lymphocytes due to that TCDD enhance inflammatory process by increased proinflammatory interleukins and generation of free radical which cause destructive change in tissue ( Calkosinski *et al.*, 2014 ), these results are in agree with ( Humadi, 2019 ) who showed histopathological changes in kidney tissue exposed to toxic material ( Acrylonitrile ) including the inflammatory cells infiltrating were also appeard mainly mononuclear cells, lymphocytes and neutrophils, these changes resulting in increased the susceptibility to toxicity and infection. dilated tubules, enlargment of glomruli with glomeruli tuft, tubular proteinaceous material and granuloma

## CONCLUSSION

Overall the results of this study consistently showed that chicken exposed to TCDD in ova injection at early incubation period causing kidney damage and destruction.

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