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Residual effects of 2,3,7,8 tetrachlorodibenzo-p-dioxin (TCDD) toxicity in liver and pathological changes of the intestine in albino male rats

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Liver damage, ulcerative mucosa, shortening villi, male rats.

ABSTRACT

This study provides the residual effects of 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD) in the liver and pathological changes of intestine albino in male rats. The present study aimed to detect the influence of TCDD in the male rats; the present study was included (118 albino male rats) divided into three groups, their age ranged from 8-9 weeks, the LD50 of TCDD (70.7 µg /kg) and the period of this study 90 days. The results showed the standard of TCDD (33.90 ppb) while the remaining amount of TCDD (3.87 ppb) in the liver tissue in the 2nd group, which was less than its amount (6.42 ppb) in the 3rd group. The pathological examination revealed in 2nd & 3rd group in intestine showed sloughing & desquamation with dark basophilic crypt (mucosa) with increased in number & size of goblet cells, irregular & shorten villi, ulcerative mucosa with necrotic villi, congested & clotted blood vessels in mucosal layer with severe mononuclear cells infiltration.

1. INTRODUCTION

The term "dioxins" refers to a group of structurally similar compounds found in the environment, acquired through the food chains, and communal mechanisms of toxicity. This group also contains polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and dioxin-similar polychlorinated biphenyls (PCBs). Although these chemical groups contain a huge number of possible congeners (75 PCDDs, 135 PCDFs, and 209 PCBs), PCBs only classify an inadequate amount of stereoisomers as harmful dioxins or dioxin-like compounds [1- 4].

Polychlorinated dibenzo-p-dioxins (PCDDs), polychlorinated dibenzofurans (PCDFs), and polychlorinated biphenyls (PCBs) are examples of polyhalogenated aromatic hydrocarbons (PHAHs) (PCBs). Depending on the location and quantity of chlorine substitutions, these chemicals can include 75 chlorinated dioxins, 135 chlorinated dibenzofurans, and 209 chlorinated biphenyls. There are 75 tricyclic aromatic compounds with one to eight chlorine atoms, including 2,3,7,8-tetrachlorodibenzo-p-dioxin (TCDD). This TCDD isomer has four chlorine atoms at positions 2,3,7 and 8. There is no indication that dioxins are biosynthetically synthesized by living beings or have any industrial use [5].

2. Materials and methods

The present experiments were applied to the Lab. animal (Albino male rats) 126 male rats were involved in this study, the age about 8-9 weeks and the weight(200-220) gms, the animals were kept in plastic enclosures in an air-trained area with heat maintained at 25 ±2 C^o, this plastic cages comprising solid-wood mark as bedding and the bedding was altered constantly to confirm a clean environment, the study expended between September 2020 to March 2021 in Diyala / College of Veterinary Medicine – the University of Diyala, Rats were given food pellets and water ***ad libitum***. The LD_{50} of TCDD (70.7 µg /kg) by up & down method (Dioxin, 1980) by using 8 rats. All rats were randomized into 3 groups and were treated for 90 days, 1st group (10 rats) acted as control group feeding on a normal rat diet, 2nd group (54 rats) administration daily orally by stomach tube 1/25 from LD_{50} of TCDD for 90 days, 3rd group (54 rats) administration daily orally by stomach tube 1/50 from LD_{50} of TCDD for 90 days.

2.1 Sample Collection

Tissue samples taken at day 90 from fresh liver and intestine were collected from the sacrificed rats directly and dipped in RNA later sterile screw-cap tubes. The liver was kept at (-8) ºC for detection of the residual of TCDD by GC technique. At the same time, the intestine was left in a 10% neutral buffered formalin solution to make a paraffin block for histopathological examination.

2.1.1 Determination of (TCDD) residual in liver by GC (Gas Chromatography)

The assay for residual of TCDD in the liver (2nd & 3rd group) after 90 days of experiment was carried out in the Ministry of Science and Technology, Baghdad, the residual of TCDD in the liver was identified using a Thermo scientific GC-2010 system with Agilent J & WDB-WAX column (31m X 0.54mm). Helium was recycled as a transferor gas at a flow degree of 1ml/min. Five µls TCDD were diluted to 1ml with dichloromethane, then 1 µl was injected in the splitless mode for 1 min. Monitored by a fragmented flow with a ratio of 1:10. GC oven temperature was detained at 45°C for 2 min then was planned from 80°C - 120°C (10°C /min). After which was kept constant at 280°C for 10 min with pressure at 100 Kpa, both the line and injection temperatures were attuned at 250°C [8]. The TCDD components were recognized by mass division patterns, and their comparative percentages were designed based on GC peak areas.

2.2 Histopathological examination

After (90) days, the animals were sacrificed by the anaesthetized intramuscular with xylazine and ketamine 10%, the specimens were taken from the intestine, and the tissues were saved in 10% formalin solution for the fix, and at that time normally processed by using the histokinette, tissue pieces were implanted in paraffin and segmented by rotary microtome, and all pieces were discoloured with hematoxylin and eosin stain [6].

2.3 Statistical Analysis

The SPSS program, Version (17) software (2010), was used to statistically analyze all of the grouped data, with one-way ANOVA being employed for group comparisons. Entirely information was described as means with standard errors (SE), and statistical significance was determined by P values of 0.05. [7].

3. Results

3.1 GC technique for residual of TCDD

The results in table (1) in the 2nd & 3rd groups showed significant $P<0.05$ increased precipitation of TCDD in liver tissue in large amounts, especially in the 3rd group.

Table (1): TCDD residual (ppb) in liver of albino male rats groups.

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groups	TCDD (ppb)
References standard (TCDD)	33.90 a
2nd group (1/25 of LD_{50})	3.87 c
3rd group (1/50 of LD_{50})	6.42 b

Values are articulated as means±SE Means with changed letters are meaningfully different (P<0.05).

3.2 Pathological examination

Control group (1st group): No important histopathological changes were observed in the 1st group in the kidney at the end of the experiment (90 days).

In 2nd group: Dark basophilic crypt (mucosa) with increased in number & size of goblet cells, superficial necrosis of irregular shorted villi (fig. 1 & 2), short necrotic villi with sloughing & degenerated mucosa & submucosa layer characterised by severe diffuse haemorrhage with oedema & mononuclear cells infiltration (fig. 3 & 4), ulcerative mucosa with necrotic villi, in another area severe diffuse haemorrhage in mucosa & submucosa with mononuclear cells infiltration (fig. 5), severe haemorrhage & congested of blood vessels in mucosa layer with another section hyperplasia of the mucosal cell, infiltration of macrophage & lymphocytes cells (fig. 6, 7 & 8).

In 3rd group: Irregular & shortened villi, in some sections joined the villi together with an increase in size & number of goblet cells (fig. 9), other mucosal section showed sloughing & desquamation with dark eosinophilic necrotic crypt (fig. 10), necrotic mucosa with sloughing in the lumen with severe haemorrhage in mucosa & submucosa (fig. 11), congested & clotted blood vessels in mucosal layer with severe mononuclear cells infiltration, in some area diffuse haemorrhage (fig. 12), chronic mucosal ulceration replacement by vascular granulation tissue (fibroblast & angioblast) with diffuse haemorrhage (fig. 13).

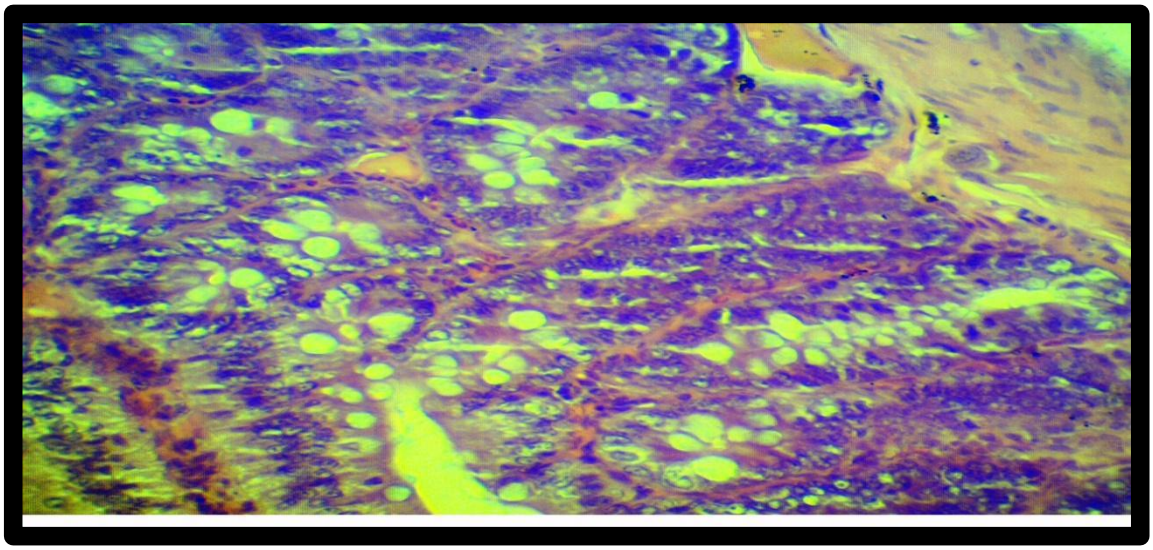

Figure (1): micrograph section in albino male rat intestine of 2nd group at day 90 showed dark basophilic villi with increased & size of goblet cells (X40; H&E stain).

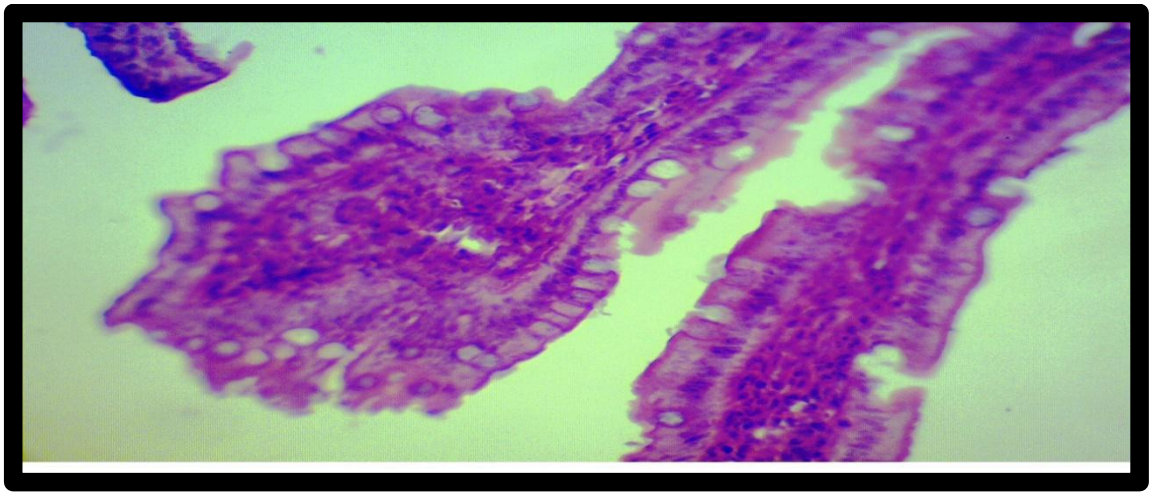

Figure (2): micrograph section in albino male rat intestine of 2nd group at day 90 showed superficial necrosis of shortened irregular villi (X10; H&E stain).

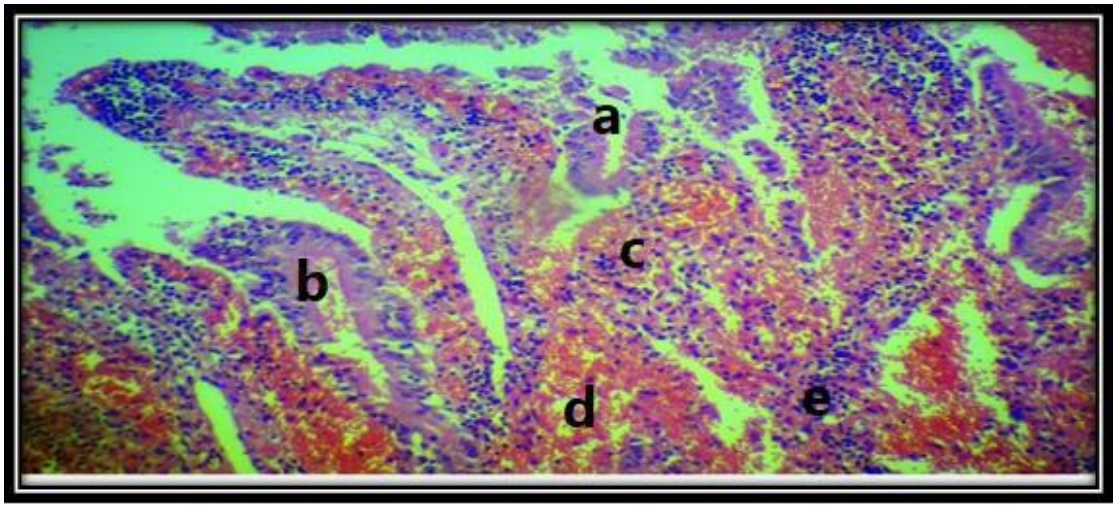

Figure (3): micrograph section in albino male rat intestine of 2nd group at day 90 showed a) sloughing & necrotic mucosa b) shorten villi c) degenerated villi d) severe diffuse mucosa haemorrhage e) severe mononuclears cells infiltrations (X20; H&E stain).

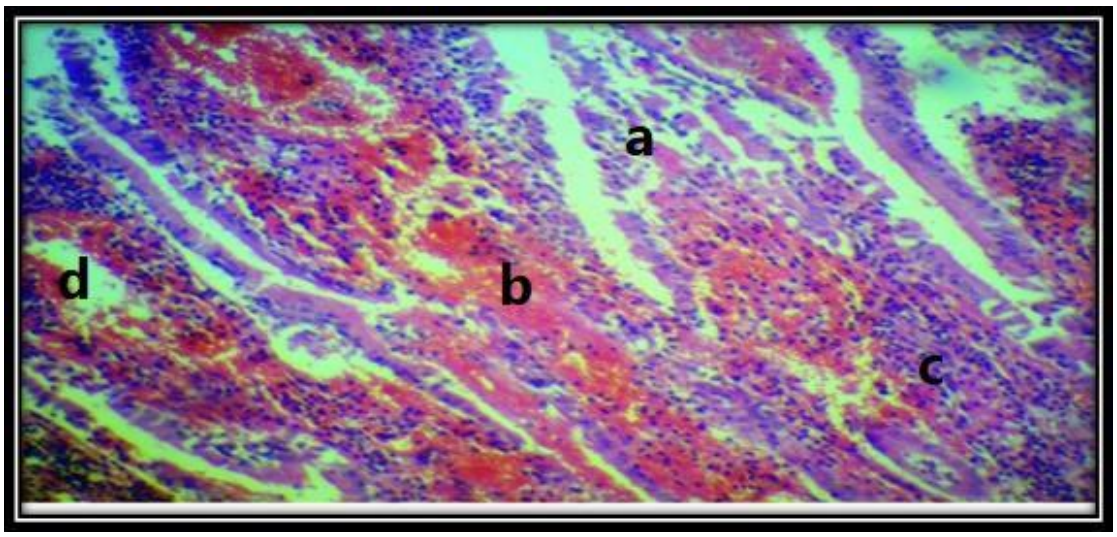

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Figure (4): micrograph section in albino male rat intestine of 2nd group at day 90 showed a) degenerated & necrotic mucosa b) severe mucosa & submucosal haemorrhage c) mononuclear cells infiltration in mucosa d) edema (X40; H&E stain).

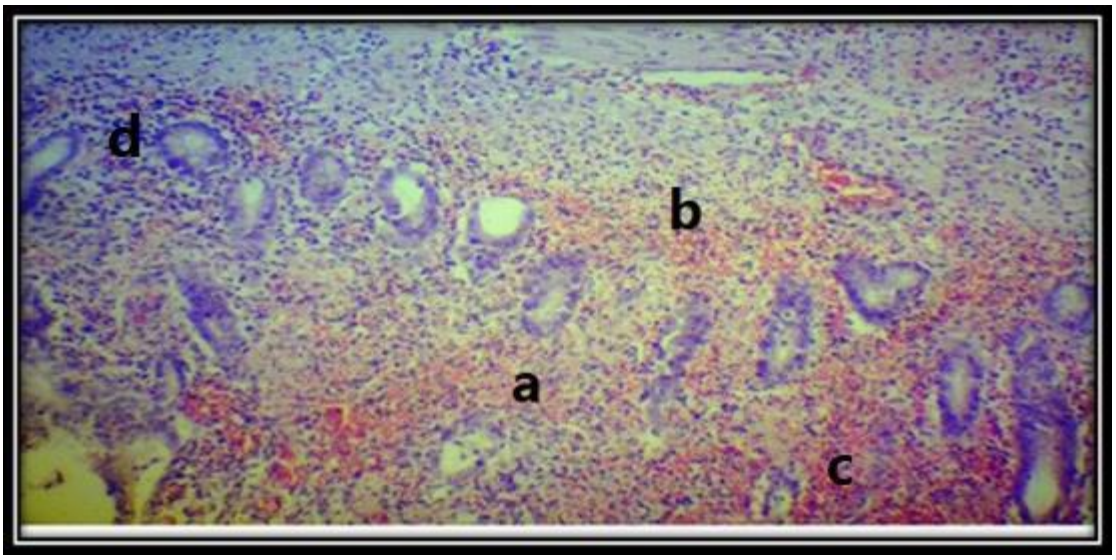

Figure (5): micrograph section in albino male rat intestine of 2nd group at day 90 showed a) ulceration b) necrotic villi c) severe diffuse haemorrhage d) mononuclears cells infiltrations.
(X20; H&E stain)

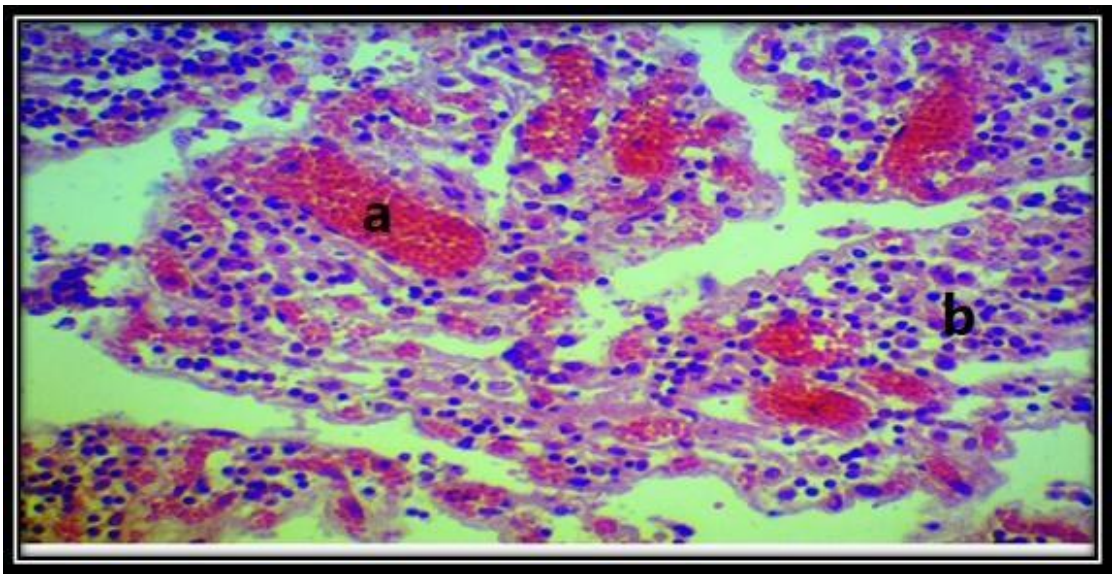

Figure (6): micrograph section in albino male rat intestine of 2nd group at day 90 showed a) severe haemorrhage & congested the mucosal in mucosal & submucosal layer b) mononuclear cells infiltration in mucosal layer.
(X40; H&E stain)

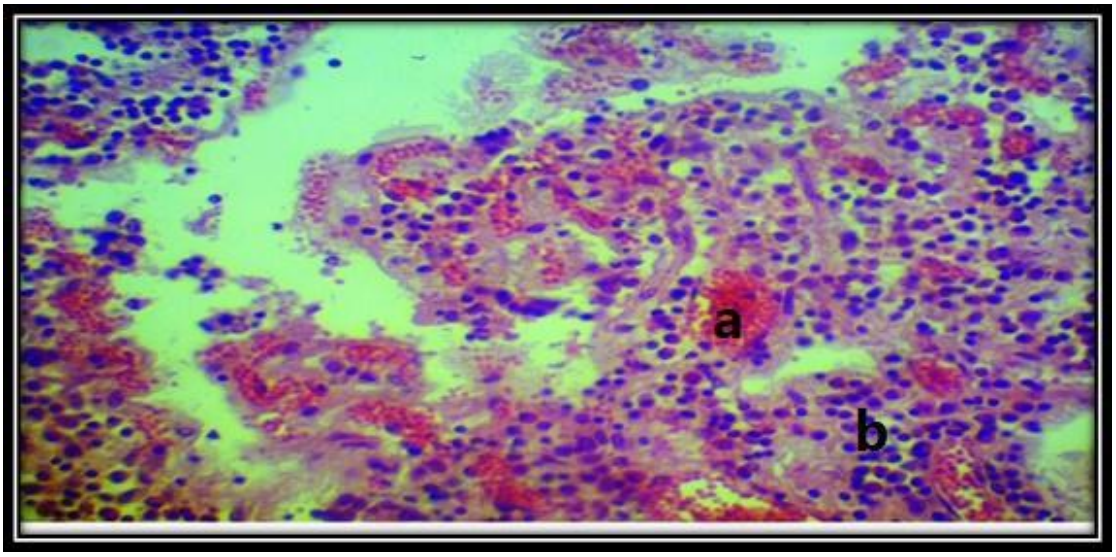

Figure (7): micrograph section in albino male rat intestine of 2nd group at day 90 showed a) severe haemorrhage & congested the mucosal in mucosal & submucosal layer b) mononuclear cells infiltration in mucosal layer.
(X40; H&E stain)

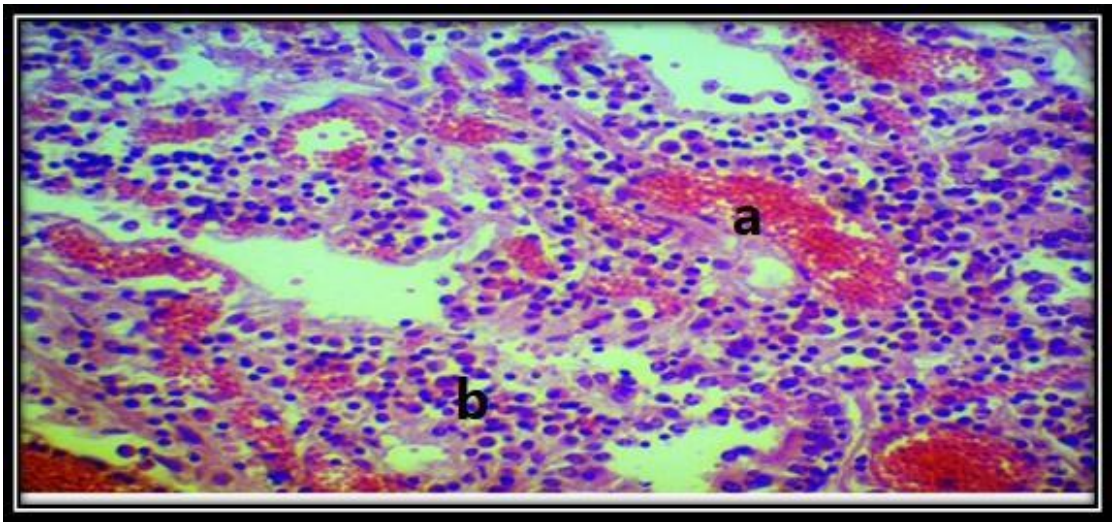

Figure (8): micrograph section in albino male rat intestine of 2nd group at day 90 showed a) severe haemorrhage & congested the mucosal in mucosal & submucosal layer b) mononuclear cells infiltration in mucosal layer.
(X40; H&E stain)

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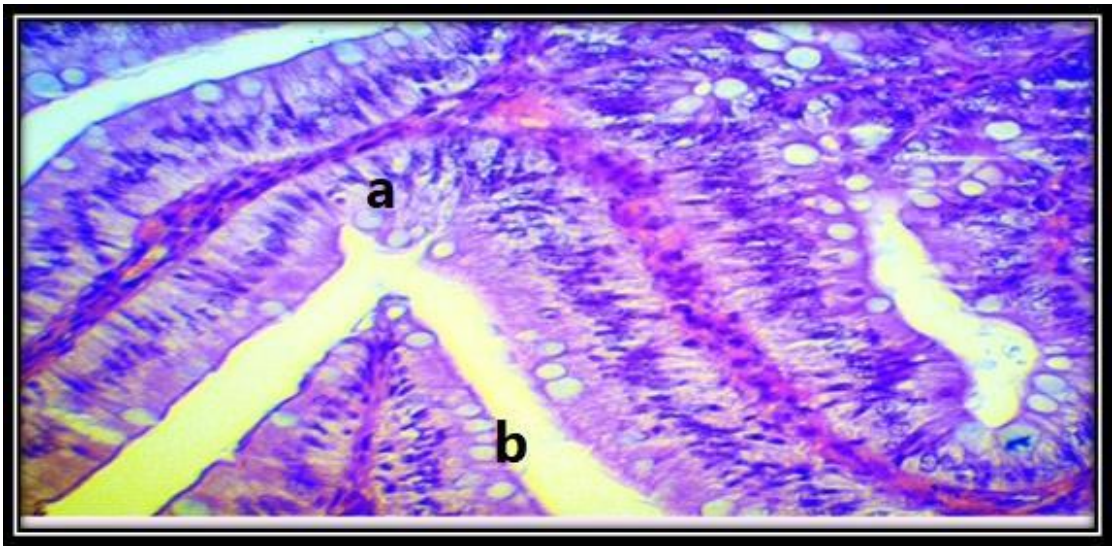

Figure (9): micrograph section in albino male rat intestine of 3rd group at day 90 showed a) irregular short joined villi b) increase in number & size of goblet cells.
(X40; H&E stain)

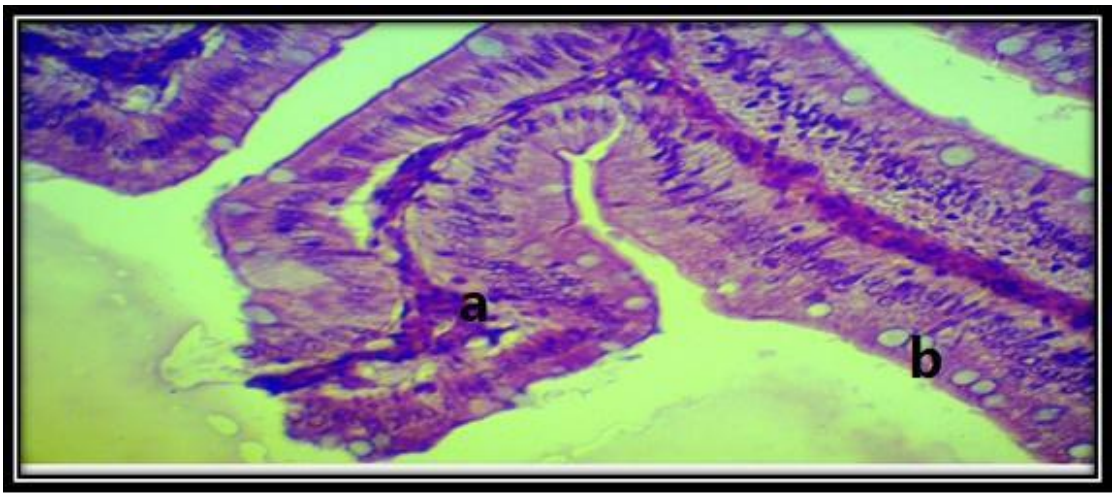

Figure (10): micrograph section in albino male rat intestine of 3rd group at day 90 showed a) dark eosinophilic necrotic villi b) irregular with increase in number & size of goblet cells.
(X40; H&E stain)

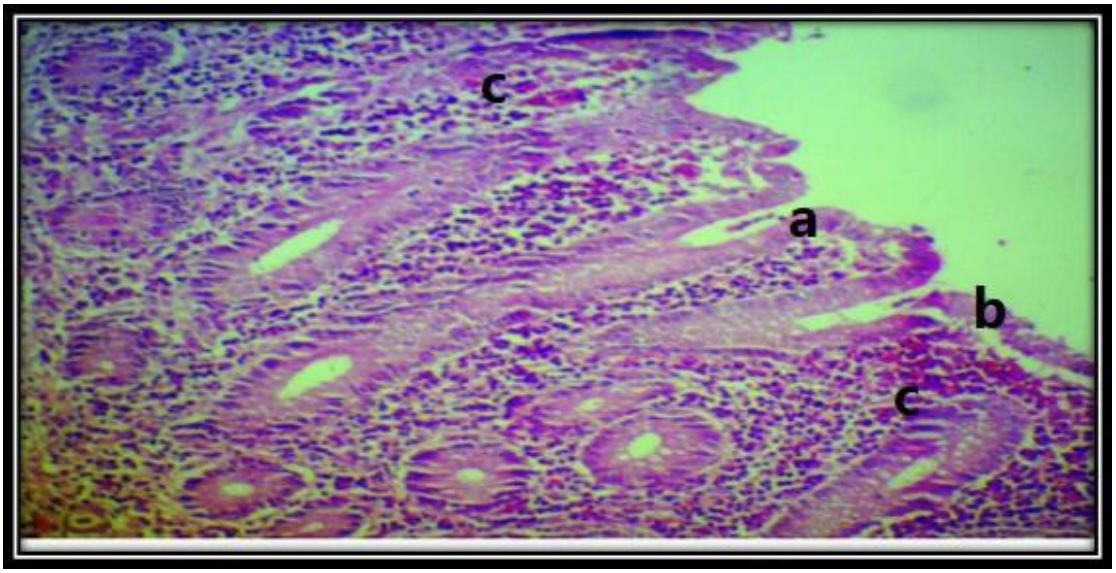

Figure (11): micrograph section in albino male rat intestine of 3rd group at day 90 showed a) necrotic villi b) sloughing mucosa c) severe mucosal haemorrhage.
(X20; H&E stain)

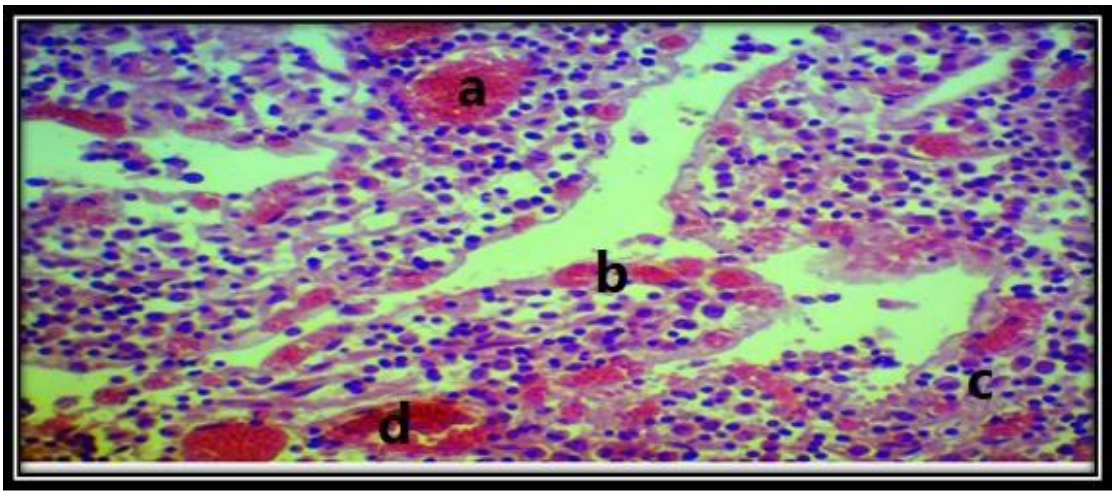

Figure (12): micrograph section in albino male rat intestine of 3rd group at day 90 showed a) clotted blood vessels b) congested blood vessels c) mononuclear cells infiltration in mucosa d) diffuse the mucosa haemorrhage.
(X40; H&E stain)

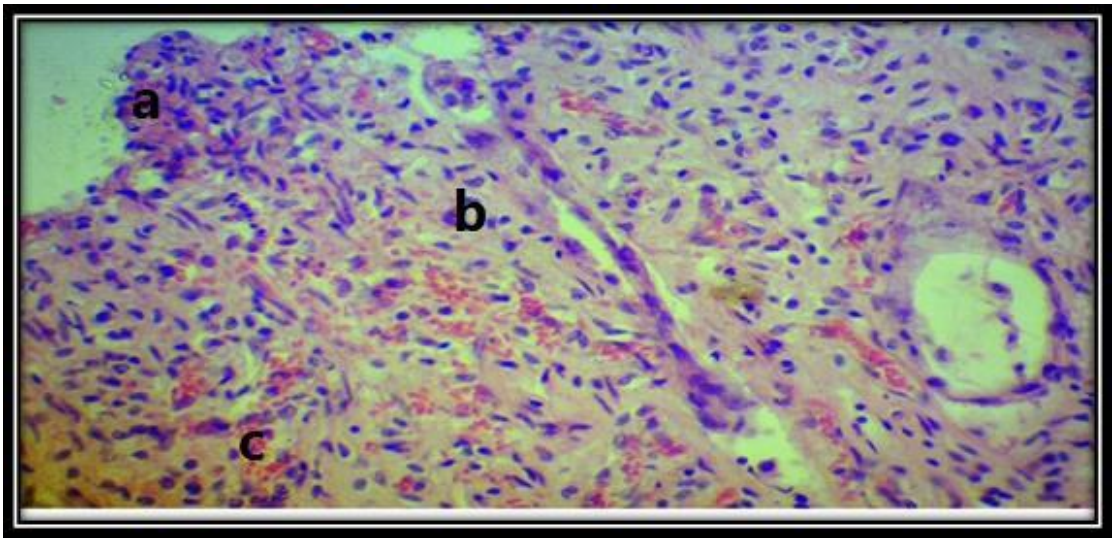

Figure (13): micrograph section in albino male rat intestine of 3rd group at day 90 showed a) chronic mucosal ulceration b) healing ulcer (granulation tissue consist of fibroblast & angioblast) c) diffuse mucosal haemorrhage
(X40; H&E stain)

4. Discussion

4.1 Residual of TCDD in liver

The result agrees that the statement is reliable with other literature inspecting inhibition activity in rat liver microsomes that cause TCDD precipitation in the liver [21]. Depending on tissue oxygen contractions, 2,3,7,8 TCDD is likely to be changed into primary & secondary metabolites after extended exposure [19] this might have instigated the detected cellular damage and cellular component breakdown, resulting in cellular necrosis on a large scale [20]. The oxidative activity of TCDD, according to [9], [17], may create ROS by many routes, including binding AhR and a decrease in membrane fluidity. The present experiment showed bigger seniority of the injured with growing exposure-outcome of TCDD into the liver cells and precipitation of TCDD. [22], [23] showed pathological effects of dioxins on the body tissue because remaining inside the body as the accumulative substance due to difficulty being processed and were gradually metabolised addicted to polar substances by drug-metabolising enzymes in the liver microsomes.

TCDD is present in entire tissues & the maximum absorptions are usually found in the liver and fat tissues, So TCDD exposures are associated with an increased risk of severely altered liver function, which can cause liver lesions [18].

4.2 Pathological changes

The findings in the second and third groups revealed extensive intestinal mucosal and submucosal haemorrhage and bleeding, as well as necrotic villi and mononuclear cell infiltration. These findings are consistent with [10], who found that the bowel lamina propria is finely equipped with mononuclear phagocytes, including both dendritic cells and macrophages, which are accountable for sampling possibly damaging antigens in In the intestinal lamina propria, the TCDD reduces macrophages and dendritic cell subtypes while increasing hepatic macrophages. Several underlying processes might be driving reciprocal alterations in these immune cell groups. First, the intestinal macrophages and dendritic cells can travel from the intestine to the liver; the liver is constantly full with blood since the intestine via the portal vein, provided that a direct link between the two tissues; and throughout situations of intestinal inflammation, such as

inflammatory bowel disease, the blood monocytes can migrate from the intestine to the liver.

The composition of intestinal contents has a direct influence on hepatic homeostasis because the liver obtains blood from the gut through the portal vein [15], [16].

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