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Skin Fibrosarcoma and Chloracne Induced by (Acute and Long Term) Effects of 2,3,7,8- Tetrachlorodibenzo-p-Dioxin (TCDD) in Male Guinea Pig (Pathological and Biochemical Assay)

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Abstract
In the present study, we investigated the effects of acute and & long term exposure orally administrated to TCDD (2,3,7,8- tetrachlorodibenzo-p-dioxin) male Hartley guinea pigs (n= 42) were randomly divided into 3 groups: 1st group (control) kept as control and given corn oil it consists 14 animals, 2nd group (n= 14) was orally administrated at dose 1 µg /kg BW by gavage TCDD mixed with 1 ml/kg BW acetone – corn oil for 7 days, 3rd group (n= 14) orally weekly administered by gavage 0.008 µg /kg BW TCDD mixed with 1 ml/kg BW acetone – corn oil for 6 months. In addition, skin samples about from 1 cm3 from neck, head & abdominal taken half of all on day 7 & after 6 months for biochemical analysis to thiobarbituric determination acid reactive substances (TBARS), catalase (CAT) and cuzen-SOD level by spectrophotometric methods, the other half skin sample were taken for histopathological analysis.
Results showed that 2nd group & 3rd group TCDD significantly ($P<0.05$) increased in TBARS & decreased in CAT & Cuzen-SOD level, pathological changes indicated at days 7 & after 6 months elevated nodules irregular on the skin of face, neck & abdomen with rough irregular dry hair & histopathological changes indicated at 7 days (acute group) chloracne acne-like lesion characterized by hyperkeratosis, acanthosis & at 6 months multinucleated giant cell with pleomorphic nuclei & fibrosarcoma tumor.
In conclusion, TCDD caused oxidative stress in a time-dependent manner & chloracne lesion mostly in acute TCDD administration & fibrosarcoma in the long term TCDD administration.
***Keywords*:** Chloracne, fibrosarcoma, guinea pig & TCDD.

INTRODUCTION

Dioxins are the dangerous & most harmful chemicals which persist in the environment for extended periods (1); they belong to the dirty dozen & called persistent organic pollutants (POPs) (2). Dioxins are a group of chlorinated organic chemicals containing polychlorinated dibenzo-dioxins (PCDDs), and polychlorinated dibenzo-furans (PCDFs) are formed of two benzene rings bonded via oxygen atoms only 17 are toxic 2,3,7,8- tetrachloro dibenzo-p-dioxin (2,3,7,8- TCDD) (3). Source of dioxins natural source & have no use; it is from forest fires & volcano activities & released as byproducts of human activities especially of industrial processes, oil or coal-fired power plants & burning of chlorinated compounds (4).
Dioxins enter via food body during ingestion then reach to circulatory system enter fat and liver cells, inside cells dioxins ties to a protein called "aryl hydrocarbon (Ah) receptor, then ties to secondary protein (Ah) receptor – nuclear translocator (Ah-rnt)" and ties to DNA strand diminish in immune system function and cause tissue disruption (5).

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TCDD can cause a developmental problem in reproductive & issues of infertility in adults to result in miscarriages, damage to the immune system, hormone imbalance, acne-like skin disease called chloracne, a hallmark of dioxin exposure [(6); (7)and (8)].

Chloracne is an acne-like disorder marked by follicular hyperkeratosis, which can include cysts and pustules, as well as hair follicle involvement, particularly in the neck and faces [(9); (10)and (11)], TCDD has also been linked to occupational chloracne in those who deal with "technical chlorophenols and other derivatives" like the pesticide 2,4,5-trichlorophenoxyacetic acid.(2,4,5-T) (12) and (5).

The IARC summarized data that the highest exposure to TCDD increases the risk of cancer & classified TCDD risk cancer & classified TCDD as carcinogenic to humans (group 1) & In experimental animals, TCDD is a multi-site carcinogen, and "the mechanism includes the aromatic hydrocarbon (Ah) receptor protein" (13).

MATERIALS & METHODS

Animals management:

Male Hartley strain guinea pigs (mean weight 250 gm) were purchased from Diyala Veterinary College, Diyala city, Iraq and kept in the animal facility of the animal house in Baghdad Veterinary College, Baghdad, Iraq. Guinea pigs were raised in metabolic cages with constant humidity & exposed to a 12:12 hrs light-dark cycle; feed & water were consumed ad libitum.

Chemicals & samples:

TCDD was purchased from (Sigma chemical com. St. Louis, Missouri, US) with the highest grade available purity > 99%, Dioxin mixed in acetone- corn oil & administered orally in volume (1 ml/kg body weight) at a single dose 1 µg /TCDD/ B.W. for acute term administered group & 0.008 µg /TCDD/ B.W. weekly dose for long term administration (6 months) (14).

Experimental groups:

Forty-two (42) male guinea pigs mature, divided equally into three groups: 1st group contain (14) animals act as control group & fed on regular rodent pellet with 1 ml/kg BW daily administered mixture acetone- corn oil orally by gavage for 6 months, 2nd group contain (14) male guinea pigs orally by gavage received 1 µg /kg BW one dose TCDD mixed with 1 ml/kg BW acetone-corn oil for 7 days (acute group), 3rd group (long term) received weekly orally (0.008 µg /kg BW) by gavage TCDD mixed with 1 ml/kg BW acetone-corn oil for 6 months.

Animals were killed with carbon dioxide gas about 14 animals in the acute group, 7 animals from control at 7 days of experiment & 14 animals in the long term group & 7 animals from the control group.

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Biochemical analysis & pathological examination:

1- Catalase (CAT) k/mg protein H2O2 enzymatic decomposition directly decreases absorbance at 24 nm (15).
2- Thiobarbituric acid reactive substances (TBARS) (16).
3- Cuzn-SOD activity (U/mg protein) Copper-zinc superoxide dismutase: Inhibition of nitroblue tetrazolium (NBT) reduction owing to O2 produced by the Xanthine/Xanthine oxidase system was used to quantify activity, The quantity of protein that inhibited the NBT reduction rate by 50% was defined as one unit of SOD activity, and the outcome was measured spectrophotometrically at 560 nm (17).

Skin samples were taken after killed from the area of the abnormal lesion from epidermis & dermis after shaving the skin & clean it by disinfecting spray of 70% ethanol about 1 cm3 piece of skin taken from neck & faces any unusual necropsy findings in the skin like lesion, abnormality, color was recorded, After being fixed in 10% neutral buffered formalin for 3 days and stained with hematoxylin and eosin, 50% of the skin samples were removed for histopathological analysis xd (18). the other 50% of skin specimens from all groups were homogenized in Teflon glass by homogenizer with 150 µg kcl 1:10 (w/v), PH: 7.4, then centrifuged at 18000 Xg (4 C0) for 30 min. for determination TBARS, CAT & Cuzn-SOD activity (19).

Statistical analysis:

The SPSS application, Version 17 software, was used to analyze all grouped data (2010) statistically. For comparisons between groups, one-way ANOVA is one of the testing procedures used. Statistical significance was defined as a P value of less than 0.05. All of the data were presented as means with standard errors (SE) (20).

THE RESULTS

The Biochemical assay:

There is a significant decrease in table 1($P<0.05$) in CAT ((k/mg protein) in the 2nd & 3rd group in comparison with the control group.

Table (1): Level of CAT (k/mg protein) in skin tissue.

groups	CAT (k/mg protein)
1st group (control group)	8.79 ± 0.44 a
2nd group (acute TCDD)	5.77 ± 0.12 b
3rd group (long term TCDD)	2.84 ± 0.32 c

1st group= 7 animals, 2nd and 3rd group= 14 animals with significantly different ($P<0.05$).

While the table (2) showed significant increase ($P<0.05$) in TBARS (nmol/g tissue) in the 3rd group in comparison with 2nd & the control group.

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Table (2): Level of TBARS (nmol/g tissue) in skin tissue.

groups	TBARS (nmol/g tissue)
1st group (control group)	138.4 ± 5.2 b
2nd group (acute TCDD)	166.1 ± 7.9 ab
3rd group (long term TCDD)	295 ± 2.2 a

1st group= 7 animals, 2nd and 3rd group= 14 animals with significantly different (P<0.05).

The table (3) showed significant decrease (P<0.05) in Cuzn-SOD (U/mg protein) activity in the 2nd & 3rd group in comparison with the control group.

Table (3): Level of Cuzn-SOD (U/Mg protein) in skin tissue.

groups	Cuzn-SOD (U/Mg protein)
1st group (control group)	367.5 ± 11.0 a
2nd group (acute TCDD)	294.0 ± 17.2 b
3rd group (long term TCDD)	208.2 ± 24.8 c

1st group= 7 animals, 2nd and 3rd group= 14 animals with significantly different (P<0.05).

Pathological changes:

Microscopic lesion of 2nd group characterized by hyperkeratosis with irregular thick eosinophilic keratin and parakeratosis with cellular infiltration of neutrophils & cystic dilation of hair follicles (fig. 1&2), skin in epidermis infected with neutrophils and follicular cyst consists of basaloid epithelial cells & sometimes keratin & lined by squamous cell epithelium with acanthosis (fig. 3,4&5), acanthotic epidermal layer prominent basophilic nuclei with necrosis & stratum corneum increased thickness of acantholytic cells with narrow, elongated thin ridges replaced mainly by fibrous connective tissue (fig. 6).

3rd group (long term TCDD) skin presented microscopically epidermal acanthosis, dermis apoptosis with inflammatory cells & granulation tissue in dermis layer (fig. 7), prominent nuclei (hyperchromatin) in irregular epidermal with infected cyst cavity of hair follicle & severe brown pigment mostly melanin present in epidermis (fig. 8), necrosis of epidermal layer with severe infiltration of mononuclear cells mostly lymphocytes, hemorrhage & dilated B.V. (telangiectasis) & thick a cellular collagen (fig. 9&10), infiltration of eosinophilic cells in dermal layer with edema & bundles of collagenous fibroblast & macrophages infiltration (11&12), severe macrophages infiltration with immature fibroblast & homogenous collagenous fiber hyperkeratosis & cystic dilation of hair follicles with squamous cell epithelium (fig. 13&14), whorls & interwoven bundle of immature fibroblast highly cellular & pleomorphic with multinucleated giant cells of bizarre, shape nuclei, nuclei of cells oval to round hyperchromatic & hemorrhage (fig. 15&16), edema with highly cellular pleomorphic nuclei varied in size fusiform & polygonal with multinucleated giant cells (fig. 17), whorls & interwoven bundle of immature eosinophilic fiber with fibrosarcoma bundles of a neoplastic fibroblast, severe vascularity & hemorrhage (fig. 18).

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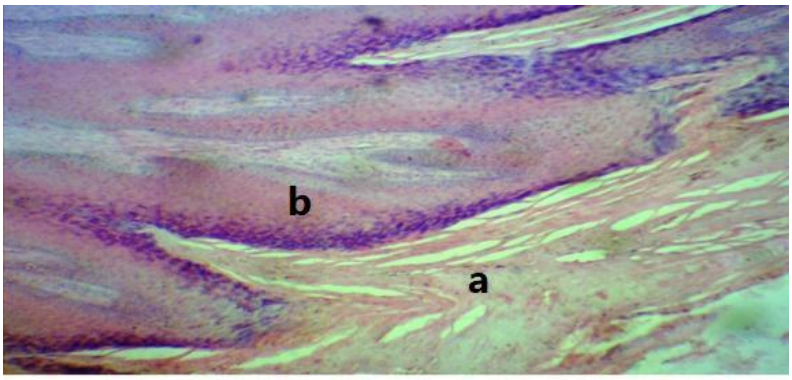

Figure (1): Microscopic section of skin in the 2nd group at 7 days showed: a) hyperkeratosis with irregular eosinophilic thick layer b) acanthosis of the dermis layer.
(X40 H&E)

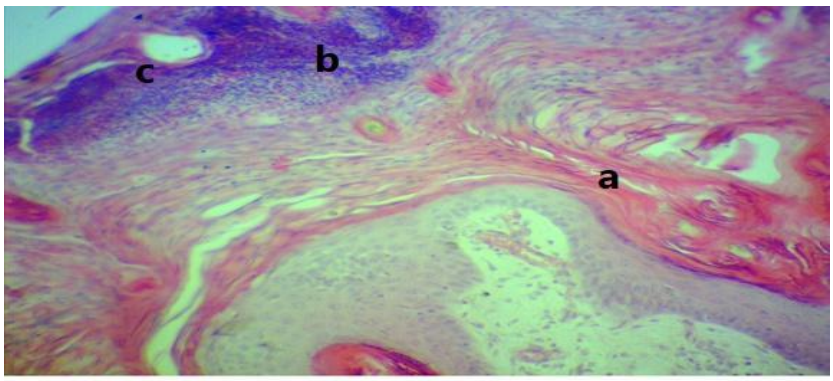

Figure (2): Microscopic section of skin in the 2nd group at 7 days showed: a) irregular eosinophilic thick hyperkeratosis & parakeratosis b) cellular infiltration of neutrophils c) cystic dilation of hair follicle.
(X40 H&E)

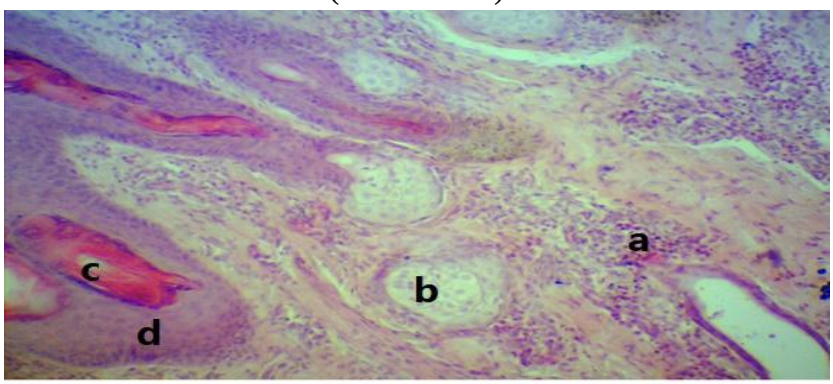

Figure (3): Microscopic section of skin in the 2nd group at 7 days showed: a) infected skin with neutrophil b) follicular cyst of basaloid epithelial cells c) hyperkeratosis with cyst cavity contain keratin lined by squamous epithelium d) acanthosis.
(X40 H&E)

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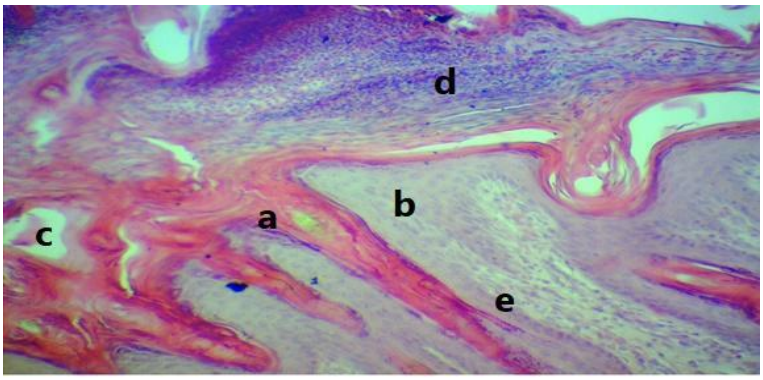

Figure (4): Microscopic section of skin in the 2nd group at 7 days showed: a) irregular hyperkeratosis b)acanthosis c) cyst cavity contains keratin d) infected epidermis with neutrophil e) ridges narrow & elongated.
(X40 H&E)

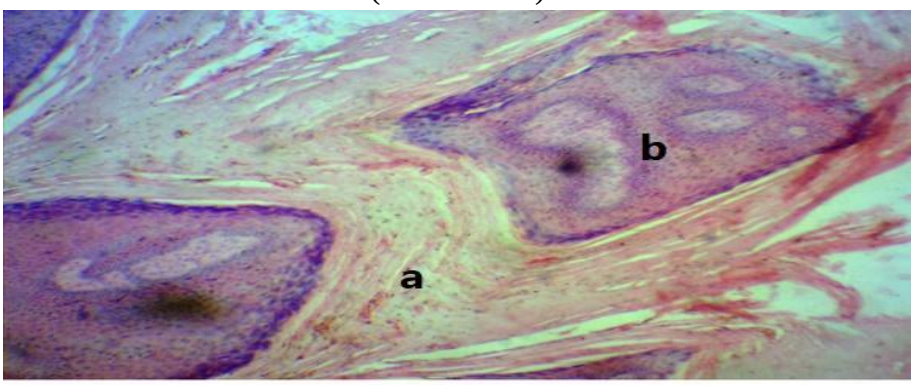

Figure (5): Microscopic section of skin in the 2nd group at 7 days showed: a) severe hyperkeratosis & parakeratosis invade epidermis b) irregular epidermis acanthosis with hyper eosinophilic cytoplasm.
(X40 H&E)

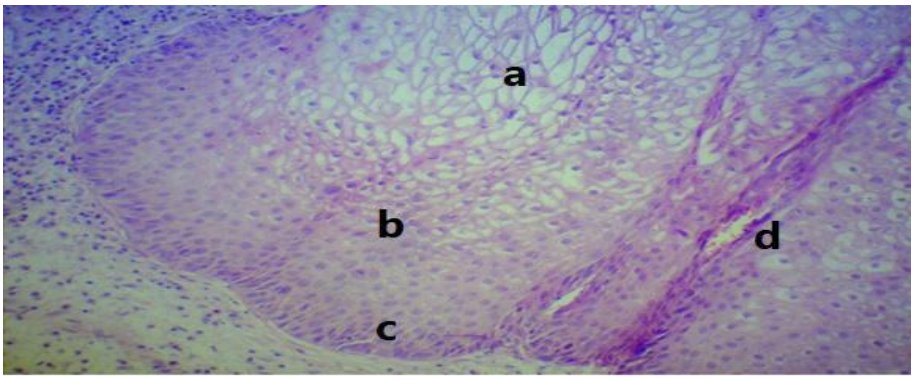

Figure (6): Microscopic section of skin in the 2nd group at 7 days showed: a) severe acanthosis b) cells in epidermal layer irregular & necrotic c) stratum corneum increased thickness acantholytic cells d) narrow elongated thin ridges mostly replaced by F.C.T.
(X40 H&E)

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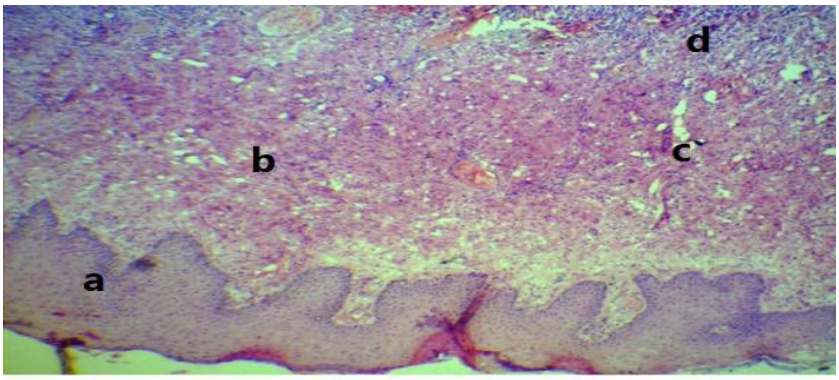

Figure (7): Microscopic section of skin in the 3rd group at 6 months showed: a) epidermal acanthosis b) dermis apoptotic cells c) granulation tissue d) inflammatory cells.
(X20 H&E)

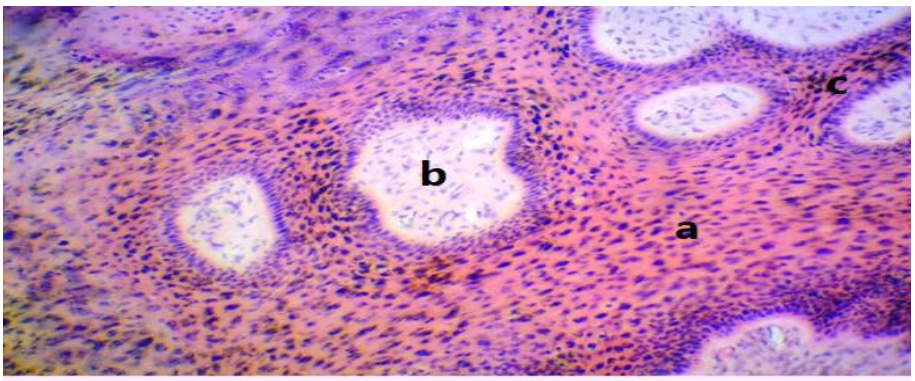

Figure (8): Microscopic section of skin in the 3rd group at 6 months showed: a) irregular epidermal hyperplasia with hyperchromasin b) infected cyst cavity of hair follicle with inflammatory cells c) severe brown mainly melanin present in epidermis.
(X40 H&E)

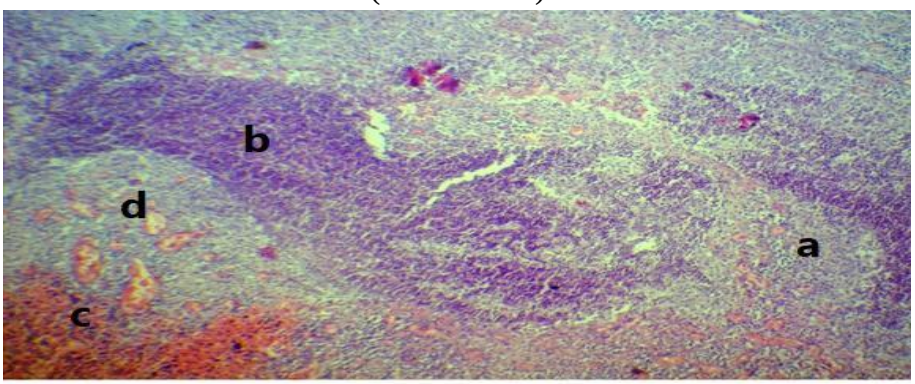

Figure (9): Microscopic section of skin in the 3rd group at 6 months showed: a) necrosis of epidermal layer cells b) severe infiltration of MNCs mostly lymphocytes c) hemorrhage d) dilated blood vessels (Telangiectasis)
(X20 H&E)

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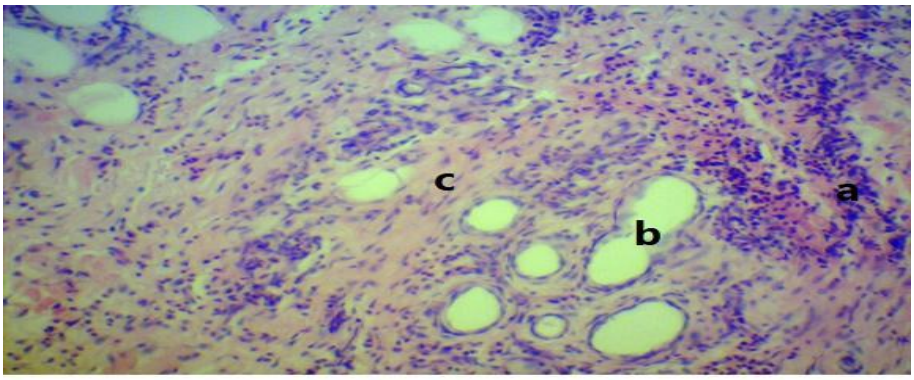

Figure (10): Microscopic section of skin in the 3rd group at 6 months showed: a) skin infiltrated with macrophages in epidermal layer b) cystic dilation of hair follicles c) thick cellular collagenous.
(X40 H&E)

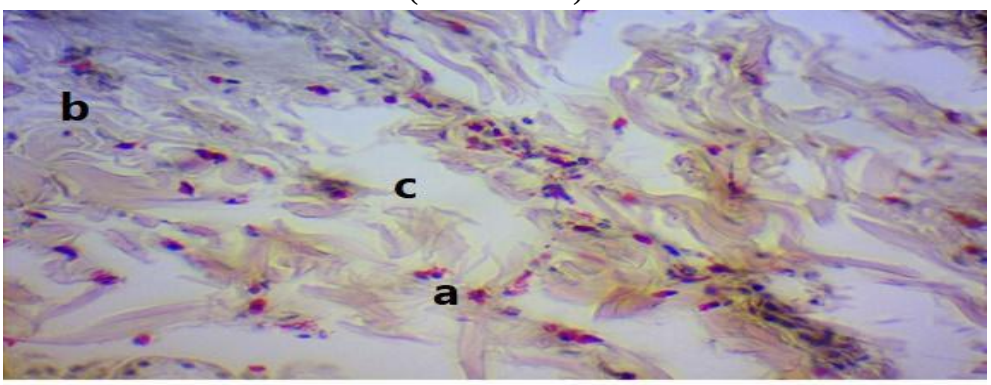

Figure (11): Microscopic section of skin in the 3rd group at 6 months showed: a) eosinophilic cells infiltration in dermal layer b) bundles of collagenous fibroblast c) edem
(X40 H&E)

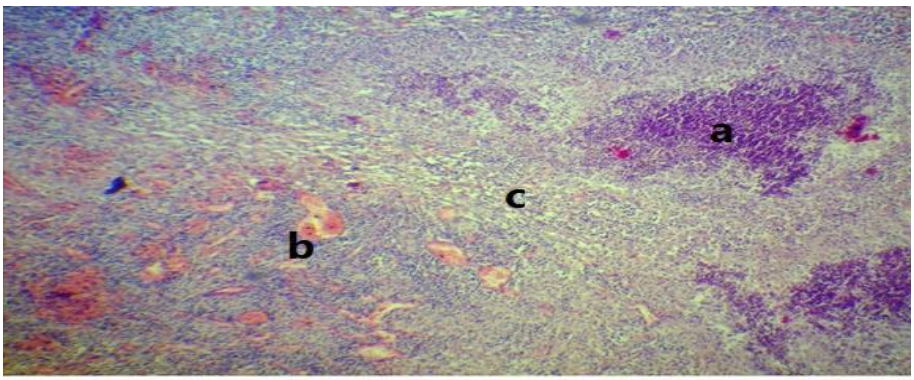

Figure (12): Microscopic section of skin in the 3rd group at 6 months showed: a) severe MNCs infiltrate in epidermal layer b) cyst-like contain keratin with squamous cell layer c) hyperchromatic & hyperplasia of epidermal layer.
(X40 H&E)

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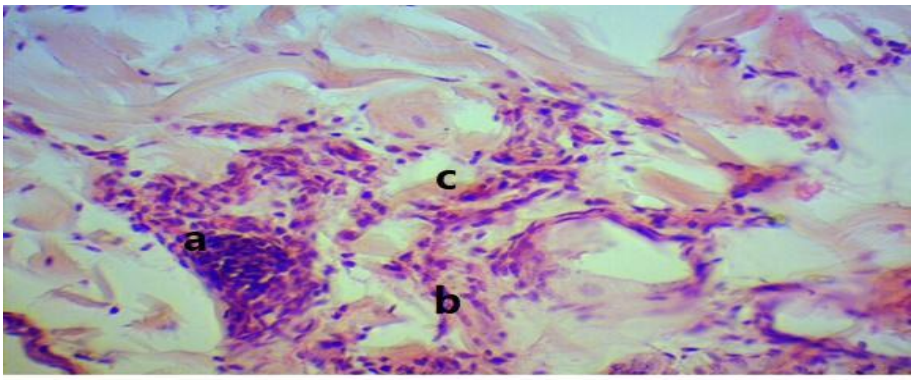

Figure (13): Microscopic section of skin in the 3rd group at 6 months showed: a) severe inflammatory cells infiltration mostly macrophages & lymphocytes b) immature fibroblast c) homogenous collagenous fiber.
(X40 H&E)

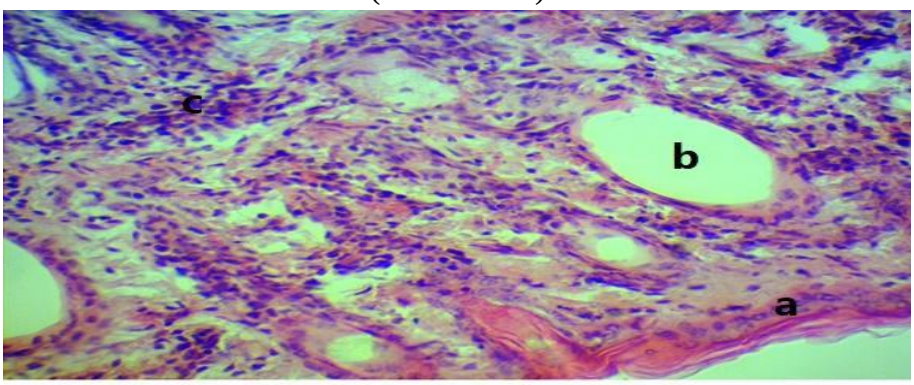

Figure (14): Microscopic section of skin in the 3rd group at 6 months showed: a) hyperkeratosis b) cyst dilation with squamous cell epithelium c) severe MNCs epidermal infiltration.
(X40 H&E)

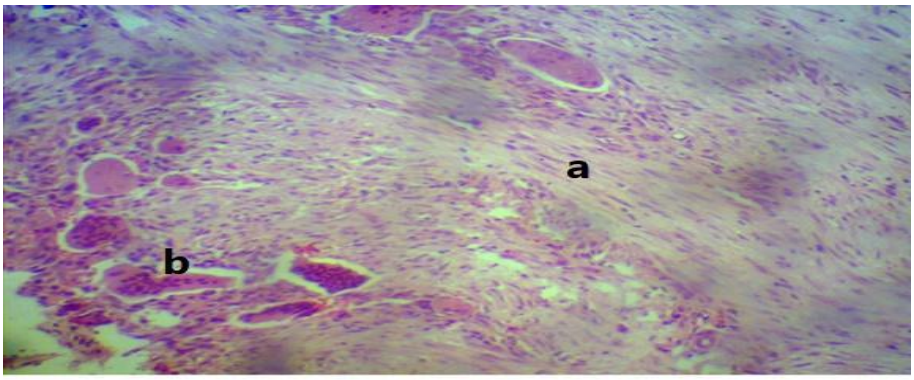

Figure (15): Microscopic section of skin in the 3rd group at 6 months showed: a) whorls & interwoven bundle of immature fibroblast highly cellular & pleomorphic b) multinucleated giant cells of bizarre shape nuclei.
(X40 H&E)

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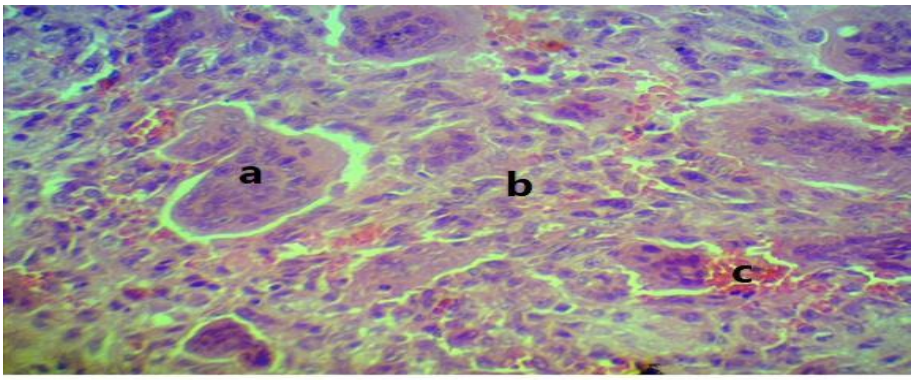

Figure (16): Microscopic section of skin in 3rd group at 6 months showed: a) multinucleated giant cells with bizarre shape b) nuclei of cells round to oval hyperchromatin c) hemorrhage. (X40 H&E)

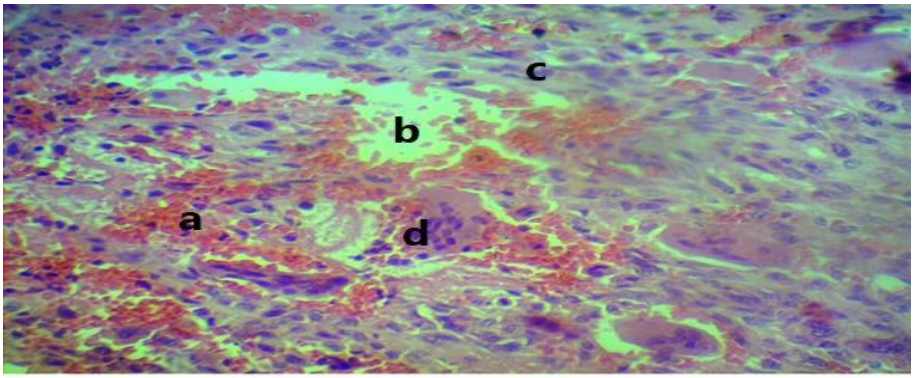

Figure (17): Microscopic section of skin in the 3rd group at 6 months showed: a) highly vascular & hemorrhage b) edema c) highly cellular pleomorphic nuclei varied in size like fusiform & polygonal d) multinucleated giant cells.
(X40 H&E)

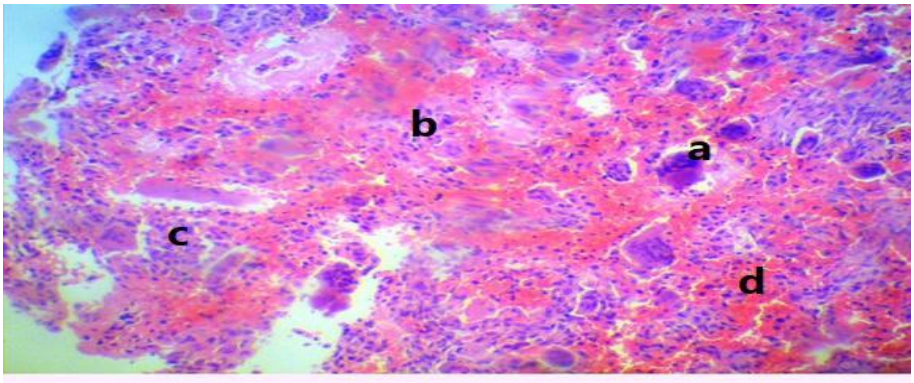

Figure (18): Microscopic section of skin in the 3rd group at 6 months showed: a) multinucleated giant cells b) whorls & interwoven bundles of immature eosinophilic fibers c) fibrosarcoma bundles of anaplastic fibroblast d) vascularity & hemorrhage.
(X40 H&E)

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DISCUSSION

In the present study, the antioxidant enzymes showed a significant decrease ($P<0.05$), especially CAT & SOD, while TBARS showed an effective increased ($P<0.05$) at 7 & highly significant in 6 months at 3rd group & thus reflecting oxidative stress induced by TCDD, these results are similar effect observed by (21) & (22). Also agree with (23), Because the Ah receptors are required for TCDD to work, the bulk of the damaging activities and actions of dioxin are dependent on the organization of this complex. As a result, TCDD does not affect live cells that lack Ah receptors(1).

Acute TCDD showed time dependent so in long term TCDD at 6 months showed highly significant decreased in antioxidant enzyme due to damage in skin tissue (24); (25), when TCDD absorbed & enter circulatory system will get up in fat & liver cell inside the cell dioxin ties to a protein called aryl hydrocarbon receptor- nuclear translocator (Ah- rnt), the Ah-rnt conveys the protein- dioxin complex into the cell nucleus & then ties to strands of DNA which can make certain genes turn on or often bring about issues causing tissue cancer (26); (27), the most pathological change at 7 days characterized by acanthosis & hyperkeratosis, follicular cyst (13); (11) and (27), these results are agree with (5) who reported pathological changes after TCDD exposure characterized by hepatic damage, jaundice, centrilobular vacuolated, hepatomegaly, abscess, hepatosteatosis, hyperplasia and multiple granuloma, summarized that acute exposure to TCDD developed chloracne which is an acne- like indicated by follicular hyperkeratosis, also these results similar to (23)During PCB exposure, liver cells contain single or multiple big lipid droplets of different sizes that shift the nucleus to the periphery of the cells, resulting in Acute hepatitis with confluent necrosis liver with centrilobular necrosis, mast cells aggregated in the liver parenchyma forming a foci causing hepatocyte atrophy and hemorrhage, liver abscess consisting of liquefactive necrosis surrounded by dead basophilic, neutrophils, and zone of fibrous connective tissue, liver abscess consisting of liquefactive necrosis surrounded by dead bas.

Long term exposure to TCDD causes multinucleated giant cells, immature collagen with fibrosarcoma; these findings are similar to those of (23) who found multiple granulomas with giant cells and calcification at day 90, indicating that TCDD causes liver damage depending on the dose and duration of exposure, and also that pleomorphism irregular epithelioid granuloma is an early indicator of liver cancer, the onset of chloracne In individuals following acute exposure & may develop within months cause tumor, the IARC summarized that highest exposure to TCDD increased risk of cancer at all sites combined & some evidence for increased risk of specific cancer such as non- Hodgkin's lymphoma, multiple myeloma or digestive system cancer (9); (10) and (11) & classified TCDD carcinogenic to human (group 1) & have multisite carcinogen in experimental animals by specific gene expression associated with toxicity by binding to Ah receptor protein which highly conserved & function in the same way.

Fibrosarcoma is a long term malignant tumor of the skin due to toxicant in humans as animals; the tissue concentration was similar in the heavily exposed, which increased cancer incidence, multinucleated giant cells is a macrophage present in chronic disease associated

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with the highly toxic compound, estimated TCDD levels increased risk cancer at 495-31800 pg/g lipid (28). The International Agency for Research on Cancer (IARC) under the world health organization (WHO) identified TCDD as the most toxic of all dioxins compounds and as human carcinogenic, sometimes not direct damage to genes but rather thought to cause promotional activities on the initiated cell damaged by other possible carcinogens (29); (5), so the long term TCDD administration caused skin fibrosarcoma.

Conclusion:

TCDD caused oxidative stress in a time-dependent manner & chloracne lesion mostly in acute TCDD administration & fibrosarcoma in the long term TCDD administration.

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