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Genotoxic Effects of Dioxins on Adult Male Rats With Special Regards to Cytogenetical and Histopathological Studies

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ABSTRACT

The aim of the present study was to identify the genotoxic effects of some of dioxins components and the histopathological changes in testis, liver and kidney of adult male rats. A total number of 40 adult male rats was used. Animals were divided into equal five groups (n= 8). Animal were injected intraperitoneally (I /P) with a single dose of dioxins (25 µg/kg body weight). The first group was kept as control, while, the second, third, fourth, fifth groups were slaughtered 1, 2, 3, 7 days after treatment with dioxins respectively. Liver and bone marrow samples were collected for DNA fragmentation and cytogenetic analysis (micronucleus test and chromosomal aberrations) respectively. Tissue specimens were taken from testis, liver and kidney for histopathological examination. Results showed non-significant difference in numerical chromosomal aberrations in all treated groups except the seven day group which showed significant changes as compared with control. There was an increase in the mean percentage of the total aberrations in rats of 3 and 7 days groups treated with dioxins (12.5 ± 0.88 and 13.00 ± 1.02, respectively) than the control (9.0± 0.91) at ($p< 0.05$). Treatment with dioxins induced a significant increase in the frequency of micronucleated polychromatic erythrocytes (MNPCEs) at 1,2,3,7 days post treatment. The degree of increase of MNPCEs is directly proportional to the days after treated with dioxins. The mean percentage of DNA fragmentation induced by dioxins was highly significantly elevated after treatment. It reached 49.13% and 54.52 % after treatment with dioxins in 3 and 7 days groups respectively. The histopathological changes were in parallel way with the cytogenetic results, in 3 days and 7 days groups. Testis showed severe degeneration, necrosis and desquamation of the different stages of the spermatogenic germ cells and Sertoli cells, in addition to decrease in the number of sperm cells in the lumen of seminiferous tubules. Hepatic tissue revealed lymphocytic hepatitis, degeneration and necrosis. Kidney showed severe congestion and hyaline casts formation.

Finally, it could be concluded that dioxins had genotoxic effects and may induce mutagenicity in bone marrow cells. Moreover, dioxins exerted pathological changes in liver and kidney tissues in addition to, it caused testicular lesions of male rats which may affect on the reproductive performance.

Key words: Dioxins, Histopathology, Cytogencity, chromosomal aberrations, micronucleated assay, DNA fragmentation.

Introduction

Dioxins containing polychlorinated dibenzo-p-dioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs) are generated naturally through processes such as forest fires and waste incineration; dioxins also are generated as byproducts of industrial processes. 2, 3, 7, 8-Tetrachlorodibenzo-*p*-dioxin (TCDD) is the most toxic, persistent and widely spread environmental pollutant compounds among polychlorinated aromatic hydrocarbons; These components causing public health hazard whereas humans are generally exposed to such compounds, which were incorporated into food, drinking water, soil, dust, smoke and air (Niittynen *et al.,* 2002 and Kwon *et al.,* 2004). Furthermore dioxins were persist in environment for a long time due to their slowly biodegradability. These compounds are deposits in adipose tissue and often excreted in human breast milk (Sharara *et al.,* 1998).

The susceptibility to TCDD among animal species was completely difference, as the LD50 of TCDD in hamsters is approximately 8000-fold higher than that in guinea pigs (Kawakami *et al.,* 2005). The World Health Organization (WHO) recommends a maximum total daily intake of 1–4 Piko Gram Toxic Equivalent Quantity (TEQ)/kg body weight (Van Leeuwen, 2000).

The induction of TCDD toxicity was greatly ruled by The aryl hydrocarbon receptor (AhR), when TCDD gets introduce to the animal tissues and binds with AhR then translocates from the cytoplasm to the nuclei, where it switches its partner molecule from heat shock protein90 (Hsp90) to AhR nuclear translocator

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(Arnt); Thus formed AhR/Arnt heterodimer binds a specific DNA sequence. This change in protein synthesis plays a key role in the pathogenesis because of its importance in all known life forms (Liu *et al.,* 2014). This pathway of (AhR) and TCDD could be uncovered the metabolic and the carcinogenic effects of TCDD which leading to gene expression alteration and tumor promotion. (Mimura and Fujii-Kuriyama, 2003). Moreover, interaction of TCDD with AhR leading to over-expression of cytochrome P450 (CYP) 1A and 1B that leading to high generation of reactive oxygen species (ROS) and liver tumor promotion (Viluksela *et al.,* 2000; Chen *et al.,* 2004; Lin *et al.,* 2004 and Moennikes *et al.,* 2004). Hepatocellular damage and cancer are the most important toxic effect of TCDD (Pohjanvirta and Tuomisto, 1994).

There were a few cytogenetic studies on dioxins toxicity performed in both humans and animals in vivo or in vitro showing chromosomal changes (Mably *et al.,* 1992; Johnson *et al.,* 1994 and El-Sabeawy *et al.,* 1998). Also Fletcher et al. (2005) suggested that even low-dose of TCDD exposure can alter the expression of several genes, indicative of cellular stress or DNA damage associated with cell cycle control. DNA damage and chromosomal abnormalities in mammalian cells are considered as a circumstantial evidence of TCDD toxicity (Iannuzzi *et al.,* 2004). DNA damage in brain tissue of mice and rats intoxicated by TCDD could be contributed to the high generation of ROS, lipid peroxidation after toxicity (Hassoun *et al.,* 2004).

Several reports showed TCDD toxicity induced pathological changes in male reproductive system which were reduction in size of testis, prostate glands and seminal vesicles, besides to decrease in the number of sperm count (Khera and Ruddick, 1973; Moore *et al.,* 1985; Rune *et al.,* 1991); Johnson *et al.,* 1992 and Kwon *et al.,* 2004). Moreover, it has also been reported that TCDD causes separation and necrosis of spermatogonia and Sertoli cells from the basement membrane of seminiferous tubules (Rune *et al.,* 1991; Kim *et al.,* 1999; and Kwon *et al.,* 2004). TCDD is known as reproductive toxicant where it causes inhibition of spermatogenesis and alteration of the testicular morphology (Mably *et al.,* 1992; Johnson *et al.,* 1994 and El-Sabeawy *et al.,* 1998). Furthermore TCDD decreases testicular production of testosterone and estrogen as well as alters regulation of pituitary luteinizing hormone secretion and both the signaling kinase activities and epidermal growth factor receptor binding in the testes (Mably *et al.,* 1992; Li *et al.,* 1997; El-Sabeawy *et al.,* 1998; Kohn, 2000 and Petroff *et al.,* 2003).

Therefore, the aim of this study was to throw light on the adverse effects of dioxin throughout clarifying its genotoxic effects on the liver and bone marrow of adult male rat by using the cytogenetic analysis. In addition to studying the histopathological changes in the testis, liver and kidney.

Materials and Methods

Dioxin standard:

The stock standard solution was obtained from Frebrug, Germany. It contained 17 native and C13 labeled 2, 3, 7, and 8-substituted polychlorinated dibenzodioxins (PCDDs) and polychlorinated dibenzofurans (PCDFs).

Lethal dose 50 (LD50) in several species of rats is 10–20 µg/kg b.wt. TCDD (Pohjanvirta and Tuomisto, 1994 and Niittynen *et al.,* 2002).

Animals and experimental design

A total number of 40 adult male rats were used in the present study. The animals were kept and reared in experimental animal house of National Research Center, Giza. The animals were fed on a standard pellet diet and water ad libitum. Animals were divided into five groups; each group consisted of 8 rats. The first group was kept as control and injected intraperitoneally with normal saline. While the other remaining groups were injected intraperitoneally (I/P) with single dose of the standard solution of dioxins (25 µg/kg body weight). The second, third, fourth, fifth groups were sacrificed by cervical dislocation after 1, 2, 3, 7 days of dioxins inoculation respectively.

Sampling:

Bone marrow, testis, liver and kidney were collected for cytogenetic analysis and histopathological studies.

1-Cytogenetic analysis:

A-Micronucleus test:

The bone marrow of 4 animals of control and all treated groups were extracted. Smears preparation was made by using fetal calf serum according to the method of Salamone et al. (1980). At least 2000 immature erythrocytes per animals scored for the incidence of micro nucleated immature erythrocytes.

B- DNA fragmentation assay:

Tissue samples from liver were used for DNA fragmentation which was carried out according to perandones *et al.* (1993). The percentage of DNA fragmentation was expressed by the formula:

DNA fragmentation % = $\frac{\text{OD of supernatant}}{\text{OD of supernatant + OD of pellet}}$

2-Histopathological study:

Tissue specimens were collected from testis, liver and kidney. The samples were fixed in 10% neutral buffered formalin for 24 to 48 hrs. Routinely processed, embedded in paraffin wax, sectioned at (5 µm) and stained with haematoxylin and eosin (H&E) for histopathological examination according to Bancroft *et al.* (1996).

3-Statistical analysis:

The obtained data were subjected to analysis of variance (ANOVA) according to Walter&Duncan (1969) and Snedecor &Cochran (1980) at probability 5%.

Results:

1-Cytogenetic analysis:

A-Chromosomal aberrations:-

As shown in Table (1). Structural chromosomal aberrations types were chromatid gap, chromatid break, deletion and centromeric attenuation. Numerical chromosomal aberrations were hypoploidy, hyperploids and polyploidy. There is non significant difference in numerical chromosomal aberrations in all treated groups except the seven day group which showed increase significant changes when compared with control (Table 1). In case of three day and seven day groups there was increase in the mean percentage of the total aberrations (12.5 ± 0.88 and 13.00 ± 1.02, respectively) than the control (9.0± 0.91) at ($p< 0.05$).

B-Micronucleus test:-Micro Nucleated Polychromatic Erythrocytes (MNPCEs):

On analysis the frequency of micronucleated cells in bone marrow cells, it was found that the treatment with dioxins induced a significant increase in the frequency of micronucleated cells at 1,2,3,7 days post treatment with dioxins as shown in Table (2)**.** The degree of micronucleated polychromatic erythrocytes is directly proportional to the days after treatment with dioxins.

I-3-DNA fragmentation:

As shown in Table (3). The mean percentage of DNA fragmentation induced by the single dose of dioxins was highly significant elevated after treatment. It reached to 49.13% and 54.52 % after treatment with dioxins in 3 and 7 day groups respectively. Also, the mean percentage of DNA fragmentation increased to reach 36.86% and 43.3 % in the 1 and 2 day groups treated with dioxins respectively.

Table (1): Mean values of different chromosomal aberrations induced in bone marrow of all experimental groups.

	Structural chromosomal Aberrations				Numerical chromosomal aberration %			Total aberration with gaps %	Total aberrations excluding gaps%
	Chromatid gaps	Chromatid breaks	deletions	Centromeric attenuations	Hypoploids	hyperploid	polyploidy		
Control group	1.50^{a} ± 0.07	0.50^{d} ± 0.10	2.00^{c} ± 0.17	4.00^{c} ± 0.25	0.50^{c} ± 0.06	1.50^{a} ± 0.06	0.50^{c} ± 0.06	10.50^{c} ± 0.96	9.00^{c} ± 0.91
One day group	1.50^{a} ± 0.08	0.50^{d} ± 0.06	2.00^{c} ± 0.18	4.50^{bc} ± 0.24	1.00^{b} ± 0.05	0.50^{c} ± 0.07	0.50^{c} ± 0.06	11.00^{c} ± 0.84	9.50^{c} ± 0.65
Two day group	1.50^{a} ± 0.05	1.50^{b} ± 0.18	3.00^{b} ± 0.20	5.50^{b} ± 0.32	1.50^{a} ± 0.07	0.50^{c} ± 0.06	0.50^{c} ± 0.09	14.00^{b} ± 0.86	10.50^{bc} ± 1.10
Three day group	1.50^{a} ± 0.06	1.50^{b} ± 0.12	3.00^{b} ± 0.18	4.50^{bc} ± 0.25	1.00^{b} ± 0.06	0.50^{c} ± 0.09	0.54^{c} ± 0.06	12.00^{bc} ± 0.77	12.50^{b} ± 0.88
Seven day group	1.00^{b} ± 0.06	1.50^{b} ± 0.12	2.50^{bc} ± 0.45	5.00^{bc} ± 0.38	1.50^{b} ± 0.08	0.50^{c} ± 0.06	1.50^{a} ± 0.06	14.00^{b} ± 0.75	13.00^{b} ± 1.02

The means followed by the same alphabetical letters were not significantly different at the probability level of 0.05.

± SE. ⟶ The results are presented as mean value

Table 2: Frequencies of micro-nucleated polychromatic erythrocytes (MPCEs) in male rat bone marrow cells in all experimental groups.

Animal groups	Total count PCEs /animal	MPCEs %
Control group	10000	0.18 ^{d} ± 0.026
One day group	10000	0.36 ^{c} ± 0.029
Two day group	10000	0.35 ^{c} ± 0.035
Three day group	10000	0.52 ^{b} ± 0.046
Seven day group	10000	0.84 ^{a} ± 0.043
LSD at α 0.05		0.108

The means followed by the same alphabetical letters were not significantly different at the probability level of 0.05.

± SE. ⟶ The results are presented as mean value.

Table 3: Mean percentage of DNA fragmentation in liver cells of male rat treated with dioxins and control groups

Control group	DNA fragmentation (%) Mean ± S.E
Control group	14. 39^{e} ± 0.890
One day group	36.86^{d} ± 0.370
Two day group	43.3^{c} ± 1.243
Three day group	49.13^{b} ± 1.123
Seven day group	54.52^{a} ± 1.411

The means followed by the same alphabetical letters were not significantly different at the probability level of 0.05.

± SE. ⟶ The results are presented as mean value

II-Histopathological findings:

Testis:

The histopathological picture of testes in the first two treated groups was nearly similar as moderate degeneration and desquamation of the spermatogenic germ cells into the lumen of the seminiferous tubules (Fig. 1).Few numbers of the seminiferous tubules showed rupture of basement membrane. Moreover, Sertoli cells appeared normal in both treated groups.

Moderate focal oedema among some of the seminiferous tubules was seen (Fig. 2), in addition to, mild oedema was observed in the subcapsular area of the tunica albugenia. Meanwhile, the three and seven day treated groups exhibited severe degree of the above mentioned histopathological alterations as severe degeneration, necrosis and desquamation of the spermatogenic germ cells and Sertoli cells (Fig. 3). Severe diffuse interstitial oedema accompanied with degeneration and necrosis of the interstitial Leydig cells was noticed. Also moderate infiltration of mononuclear inflammatory cells mainly lymphocytes were found enclosed into the infiltrated oedematous fluid in the interstitial space (Fig. 4). In general sperms were scanty in the lumen of almost of seminiferous tubules in the all treated groups.

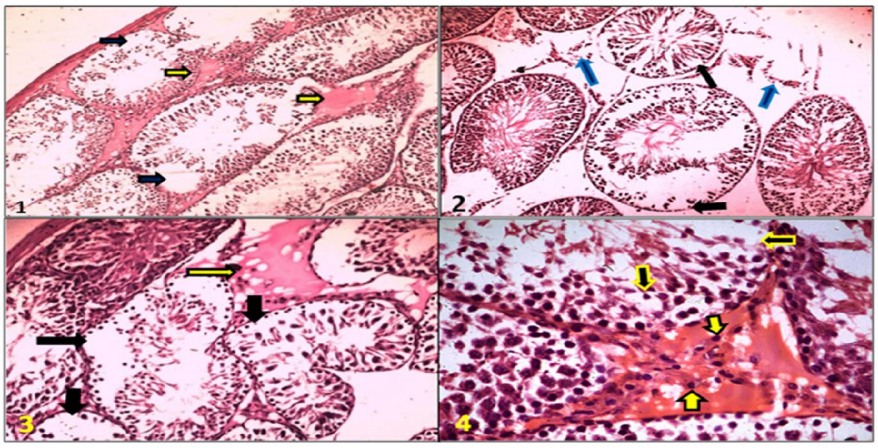

Fig. 1: Testis of rat one day post I/P injection of dioxins at a dose of 25 µg/kg body weight showing moderate degeneration and desquamation of the spermatogenic cells into the lumen of the seminiferous tubules (black arrows), moderate interstitial oedema among the seminiferous tubules. (yellow arrows). (H&E stain, X 100

Fig. 2: Testis of rat two days post I/P injection of dioxins at a dose of 25 µg/kg body weight showing severe degeneration and desquamation of the spermatogenic cells of some of the seminiferous tubules into the lumen (black arrows). Note degeneration and necrosis of Leydig cells (blue arrows). (H&E stain, X 100).

Fig 3: Testis of rat three days post I/P injection of dioxins at a dose of 25 µg/kg body weight showing severe degeneration, necrosis and desquamation of large number of Sertoli cells of the seminiferous tubules (black arrows), moderate interstitial oedema (yellow arrow). (H&E stain, X 200).

Fig. 4: Testis of rate seven days post I/P. injection of dioxins at a dose of 25 µg/kg body weight exhibiting interstitial oedema and moderate lymphocytic infiltration (yellow arrows) in addition to degeneration and necrosis of some of spermatogenic cells and Sertoli cells in the seminiferous tubules (black arrows). (H&E stain, X 400).

Liver:

The main pronounced histopathological change in one day and two day groups was moderate hydropic degeneration in the hepatocytes. Some cases showed mild sporadic lymphocytic infiltration in between hepatic cords (Fig. 5). In the two treated group the severity of degeneration was evident. Moderate focal aggregation of lymphocytic infiltration was seen (Fig. 6). In the three day and seven day treated groups, the histopathological changes become more pronounced and severe as diffuse degeneration and necrosis were observed. The hepatocytes appeared swollen with round outline and deeply basophilic pyknotic nuclei. Severe congestion in some of blood sinusoids was noticed (Fig.7 & 8).

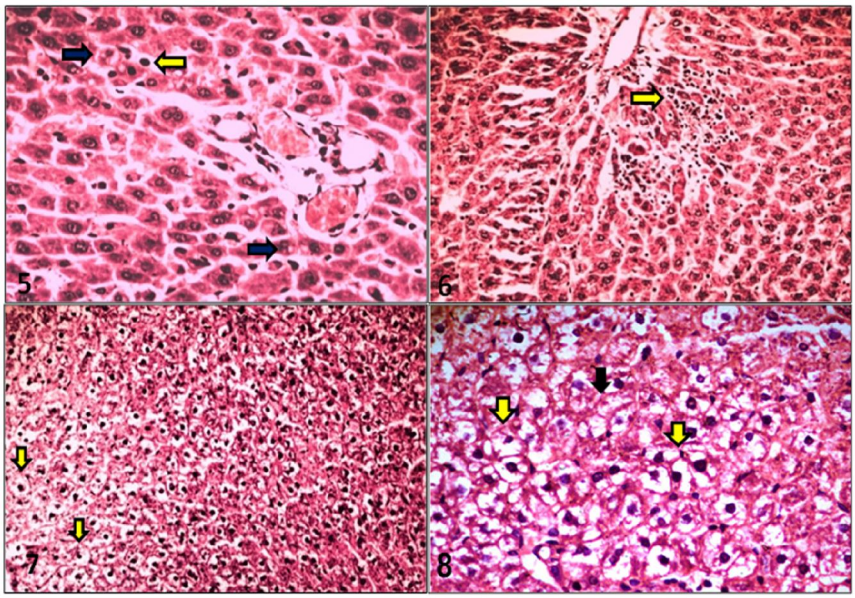

Fig. 5: Liver of male rat one day post I/P injection of dioxins at a dose of 25 µg/kg body weight showing mild vacuolar degeneration in hepatocytes (Blue arrows) accompanied with mild sporadic infiltration of mononuclear inflammatory cells mainly lymphocytes in the inter cellular space (yellow arrow). (H&E stain, X 400*)*

Fig. 6: Liver of male rat two days post I/P injection of dioxins at a dose of 25 µg/kg body weight showing moderate focal aggregation of lymphocytic infiltration in between degenerated and necrosed hepatocytes and in the periportal area. (H&E stain, X 200).

Fig. 7: Liver of male rat three days post I/P injection of dioxins at a dose of 25 µg/kg body weight exhibiting severe degeneration and necrosis of most of hepatocytes (yellow arrows) (H&E stain, X 200.

Fig. 8: Liver of male rat seven days post I/P injection of dioxins at a dose of 25 µg/kg body weight showing severe and diffuse degeneration and necrosis of hepatocytes (black arrow) The hepatocytes appeared swollen with round outline and deeply basophilic pyknotic nuclei (yellow arrows). (H&E stain, X 400).

Kidney:

The one day and two day treated groups showed vacuolar degeneration in some of the proximal and distal convoluted tubules. Some of the renal glomeruli appeared shrunken and atrophied (Fig. 9 & 10). On the other hand, the three day and seven day groups showed severe and diffuse vacuolar degeneration in most of proximal and distal convoluted tubules accompanied with formation of hyaline casts in in the lumen of some of proximal convoluted tubules. Severe necrosis of some of renal glomeruli was observed. Moreover, there was severe congestion of most of renal blood vessels (Fig. 11 & 12).

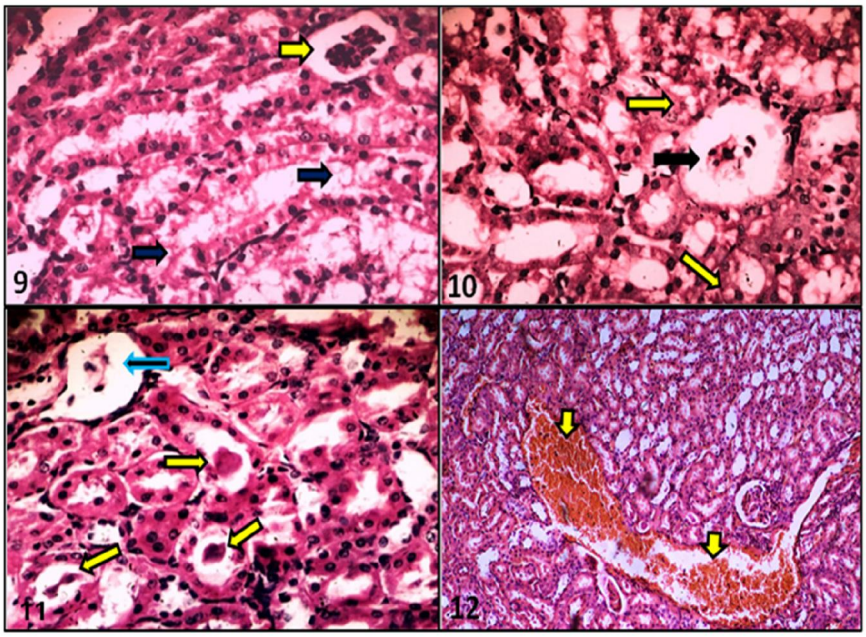

Fig. 9: Kidney of male rat one day post I/P injection of dioxins at a dose of 25 µg/kg body weight showing vacuolar degeneration and necrosis of the lining epithelium of proximal convoluted tubules (blue arrows) accompanied with shrinkage of some of renal glomeruli (yellow arrow). (H&E stain, X 400*)*

Fig. 10: Kidney of male rat two days post I/P injection of dioxins at a dose of 25 µg/kg body weight showing moderate vacuolar degeneration in some of proximal convoluted tubules (yellow arrows) accompanied with necrobiotic changes of some of renal glomeruli (black arrow). (H&E stain, X 400).

Fig. 11: Kidney of male rat three days post I/P injection of dioxins at a dose of 25 µg/kg body weight exhibiting presence of homogenous eosinophilic hyaline mass in the lumen of tubules (yellow arrows) and severe necrosis of some of renal glomeruli (black arrow). (H&E stain, X 400).

Fig. 12: Kidney of male rat seven days post I/P injection of dioxins at a dose of 25 µg/kg body weight showing severe dilatation and congestion of renal blood vessels (yellow arrows) (H&E stain, X 100.

Discussion:

It is a great important to put in our concern that exposure to environmental pollutants can produce major pathological effects in the reproductive systems of both humans and animals. The most environmental toxic contaminants known all over the world are the PCDDs and PCDFs which induce reproductive abnormalities (Gray *et al.,* 1997; Faqi *et al.,* 1998; Chia, 2000 and Lee *et al.,* 2007). TCDD was investigated for genotoxic potential in vitro using cultured mammalian cells. No evidences of unscheduled DNA synthesis were observed in cultured human cell; however, gene mutation was observed in mouse lymphoma cells and sister chromatid exchange in Chinese hamster ovary cells (ASTDR, 1998).

TCDD induced localized and discontinuous change in chromatin structure (Okino and Whitlock, 1995). This could explain the increasing number of gaps, chromatid and chromosome breaks, fragments and sister chromatid exchanges (SCEs) in the cells of exposed animals to dioxins (Elferink *et al.,* 2001; Iannuzzi *et al.,* 2004 and Farghaly *et al.,* 2009). The obtained results of this study suggested that the single dose of dioxins induced mutagenic effect in bone marrow cells of male rats. Also, it demonstrated that the degree of chromosome damage induced by dioxins related to its mutagenic effect in bone marrow cells of male rats (Inui *et al.,* 2014).

The present work indicated that dioxins induced a significant increase in the frequency of micronucleated cells in all treated groups at 1,2,3,7 days post injection. A micronucleated cells (MN) formed during the metaphase and anaphase transition of mitosis. It may arise from a whole lagging chromosome or an acentric chromosome fragment detaching from a chromosome after breakage which do not integrate in the daughter nuclei. Perucatti *et al.* (2006) found that the high chromosome fragility in the exposed sheep is only related to single high dose of dioxins, let us to consider that; dioxins may damage the chromosomes.

The results of DNA fragmentation indicated that the mean percentage of DNA fragmentation induced by single dose of dioxins was highly significant at 7 days after treatment with dioxins. Lin *et al.* (2007) noted that increase in the number of DNA strand breaks in human breast cancer cell lines (MCF-7 and MDA-MB-231) cells exposed to TCDD as measured by the single – cell gel electrophoresis (Comet) assay. Overall, this evidence confirms that TCDD induces decrease in intracellular NADP H and NAD^+ through poly (ADP-ribose) polymerase-1 (RARP-1) activation mediated by formation of DNA strand breaks. TCDD induces oxidative stress and DNA damage in human estrogen receptor α (ERα) (+) /MCF-7and ERα (-) /MDA-MB-231 breast cancer cells and whether this is accompanied by the initiation of DNA repair events. TCDD –induced oxidative stress and DNA damage may, in part, contribute to TCDD- induced carcinogenesis (Lin *et al.*, 2007). The toxic effect of dioxins seems to be mediated through the aryl hydrocarbon receptor (AhR), which plays a central role in dioxins hepatotoxicity that AhR responsible for regulation of xenobiotic metabolism, and hepatovascular development (Fletcher *et al.,* 2005).

Finally, cytogenetic results in the present study indicated that dioxins exhibited significant increase in the frequencies of micronucleated polychromatic erythrocytes (MNPCEs), the mean percentage of DNA fragmentation and the mean percentage of the total aberrations as compared with control. This means that dioxins may have a mutagenic activity in bone marrow cells and hepatotoxicity on liver cells of male rats.

Our histopathological findings in testis showed decrease in the presence of testicular sperm cells in most of seminiferous tubules; this finding could be explained by the fact that dioxins toxicity are associated with seminiferous tubule damage which induced maturation arrest in the testis (El-Sabeawy *et al.,* 1998 and Lee *et al.,* 2007). TCDD toxicity also decreases testicular production of testosterone and estrogen as well as alters regulation of pituitary luteinizing hormone secretion and both the signaling kinase activities and epidermal growth factor receptor binding in the testes (Mably *et al.,* 1992; Li *et al.,* 1997; El-Sabeawy *et al.,* 1998; Kohn, 2000 and Petroff *et al.,* 2003). The degeneration, necrosis and desquamation of Sertoli cells in the lumen of seminiferous tubules in the different groups of the current study especially in the three days and seven days groups treated with dioxin come in agreement with (Rune *et al.,* 1991; Kim *et al.,* 1999 and Kwon *et al.,* 2004) who reported that TCDD caused severe testicular damage led to the existence of Sertoli cells with few germ cells. Thus, it was suggested that the toxicity of TCDD in the testicles caused abnormal spermatogenesis.

TCDD is considered as strong hepatotoxic agent, our study confirmed this fact whereas liver of treated rats suffered from severe degeneration and necrosis of hepatocytes and also accompanied with moderate to severe lymphocytic infiltration especially with the prolongation of time of TCDD administration in the three days and seven days groups. Such changes could be explained on the basis of the metabolic pathway of dioxin; As it had a great affinity to the aryl hydrocarbon receptor (AHR) which mediates most, if not all, of these effects (Schmidt and Bradfield, 1996 and Carver *et al.,* 1998). The binding of dioxin to the AHR results in translocation of the receptor complex to the nucleus, where the receptor dimerizes with another protein known as AHR nuclear translocator protein (AHR-ARNT) (Schmidt and Bradfield 1996 and Pande *et al.,* 2005). In the nucleus, the AHR-ARNT heterodimer binds to the genome "dioxin-response element" and up regulates genes encoding a battery of enzymes, including the cytochrome P450-dependent mono-oxygenases which responsible for interleukins activity (IL) mainly IL-1 which is a soluble factor that are known to participate in chemically-

induced liver injury (Schumann and Tiegs, 1999; Yin *et al.,* 1999 and Pande *et al.,* 2005). Also another study carried out by Kurachi *et al.* (2002) who investigated that TCDD can induce gene expression changes in mouse liver 7 days after treatment with dose of 20 Ag/kg body weight. These previous studies confirmed the complicated nature of the action of TCDD on liver cells (Fletcher *et al.,* 2005).

The histopathological changes of the kidney showed an ascending degree of severity which began as mild hydropic degeneration in one day treated group until reached to severe degeneration, necrosis, and hyaline cast formation accompanied with severe congestion in the three day and seven day groups. These changes more concentrated mainly in the proximal convoluted tubules that come in accordance with (Foster *et al.,* 1986 and Lock and Reed, 1998) who found that TCDD is distributed unevenly in the kidney with the highest concentration in the outer zone of the medulla in the proximal not the distal tubular cells in the adult rats. A possible reason for this difference could be due to the formation of CYP1A1 which induced by TCDD in the kidney is region-specific with the kidney development, and that region including the thick ascending limb of Henle's loop may be very sensitive to TCDD exposure (Nishimura *et al.,* 2006).

Finally, it could be concluded that dioxins had genotoxic effects and may induce mutagenicity in bone marrow cells. Moreover, dioxins exerted pathological changes in liver and kidney tissues in addition; it caused testicular damage of male rats which may affect on the reproductive performance.

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