Pathological and hormonal effects of 2,3,7,8- Tetrachlorodibenzo-P-Dioxin (TCDD) versus antioxidant activity of Curcumin In Sprague Dawley Male Rats

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Abstract:

Current experiment was designed to evaluate the chronic effects of 2,3,7,8- Tetrachlorodibenzo-p-dioxin (TCDD) intoxicant effects and Curcumin antioxidant effects on pathological and hormonal assay in Sprague Dawley male rats at day 90. Thirty male rats divided randomly and equally into 3 groups, control group (negative control) received only normal pellet and corn oil, 2nd group (positive group) act as TCDD group received orally weekly for 90 day by stomach tube (2 µg / kg), while 3rd group received weekly by stomach tube (2 µg / kg) TCDD with (100 mg / kg) daily Curcumin for 90 day. Testosterone hormone and sperm analysis (viability, number and abnormality) assay significantly decrease ($P<0.05$) at 2nd group when compare with control and 3rd group , while pathological changes indicated at 2nd group testicular carcinoma characterized by pleomorphic and polyhydral of leydig cells and sertoli cells.

Keyword: TCDD, Curcumin, Sprague Dawley male rats.

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Introduction:

Our environment contain potential chemicals causing endocrine disruption and alter development of mammalian characteristic (1), TCDD is predominant pollutant and carcinogens even in lower doses it act mostly on the developmental and reproductive toxicity (2). In most cells line studies TCDD cause inhibit estrogen induced response (3). In testis murine cells TCDD induced process principally mediated by cystosolic aryl hydrocarbon receptor (AhR),So TCDD act directly on testicular function and morphology these including impairment of leydig cells function and decreased testicular steroidogenesis, testosterone concentration and production of sperms (4,5,6 and 7).

The TCDD its effective persistent due to its lipophilic action and slow metabolism and excretion due to its half-life in rats 3-4 weeks (8). Most studies investigating to reproductive effects of exposure to dioxin, in human have involved paternal exposure to doses, almost are limited in their ability to detect any increase in specific birth defect and alteration in hormonal level and sperm characteristics (9 and 10).

Curcumin is the pigment which give yellow color to turmeric derived from the

Curcuma Longa and used as a spice in curries, food additive and also as dietary pigment (11). Many plants have protective effects on oxidative stress and can successfully used against TCDD toxicity (12). Therefore the aim of the current study to indicated the protective effect of curcumin against TCDD toxicity in male rats reproductive system.

Materials and methods:

The TCDD (purity 100%) was obtained from Sigma chemical company (Netherlands) and were of analytical grade available, Curcumin obtained from Promega company (USA).

Animals and antioxidant treatment:

Thirty (30) healthy male Sprague Dawley rats the age 3-4 months old and body weight (300-310) gm were selected and obtained from Al-Razi Center Research, Baghdad, Iraq and housed in polypropylene sterilized cages for rats in 12 hrs. light dark cycle and 21 C^o ambient temperature drinking water and rat pellet diet were given ad libitum, all current experiment were under animal ethics guidelines of Institutional Animals Ethics Committee.

Sprague Dawley rats were divided randomly and equally into three groups (n = 10) in each group, 1st group act as control group (negative control) received only normal pellet and corn oil, 2nd group (positive) control act as TCDD group (TCDD stock solution was dissolved in acetone and diluted freshly with corn oil) and administrated to rats after evaporated the acetone under nitrogen , this group received orally weekly by stomach tube (2 µg / kg) for 90 days, while 3rd group received weekly by stomach tube (2 µg / kg) TCDD with (100 mg / kg) daily Curcumin for 90 days (13 and 14).

All animals was sacrified at 90 days, postmortem examination and blood samples were collected from heart puncture in test tube to measured testosterone hormones level while semen from the tail of epididymis was collected immediately to analysis by slicing and rinsing pressing technique then in normal saline at 37 C^o (15).

Hormonal assay:

Testosterone hormone:

After blood collection and centrifuged, serum was removed and kept at(-20 C^o) until assay, testosterone kit RIA technique, testosterone kit were obtained from Giemsa company, Germany (Euro-Diagnostica) about fifty microliter serum sample from all groups were pipetted into coated labeled tubes and 500 µl testosterone, 125 reagent were added to each tubes, then gently racks of tubes and with hand shaking for 0.5 minutes, then placed in water bath 37 C^o for one hour, all tubes aspirated and counted for 1 minute in gamma counter, the average of double measurement were carried out and results were reported in nanogram / milliters.

Sperm analysis:

a) Sperm number (dead and live) (16).
   A drop of nigrosine – eosinstain with a drop from sperm suspension mixed together on glass slide and covered by cover slip, from each sample two smear were done 200 sperm from each glass smear under light microscope (X 400) power examined as following:
   Sperm viability % = number of sperm alive / total number of sperm X 100
b) Sperm morphology (17)
   1 ml 0.9% Nacl rinsed duct deferens, then obtained sperm suspension, stained with 2% eosin to determine the percentage of abnormal sperm morphology. 200 sperms / animal were examined under light microscope (X 400) and score abnormal head and deformities.
c) Semen counting (18)
   On a warm glass slide a drop of sperm suspension placed at 37 C^o were covered by cover slide and examined under objective X 400 light microscope were examined.

Pathological examination:

The testes at day 90 of all animals groups were removed gently after recorded any abnormality in size, color, position and adhesion, then immediately impeded in Bouins solution fixative for 2-3 days and embedded in paraffin and sectioned at 5 µm, then stained by hematoxylin and eosin stain for histopathological examination (19).

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 ± standard error (SE). (20).

Results:

a) Biochemical assay:

The testosterone hormone concentration reported in 2nd group significant decreased P<0.05 when compared with the concentration of 3rdand control group, table (1).In current study the 3rd group of antioxidant (Curcumin) at dose 100 mg / kg for 90 day cause significant unchanged in hormonal concentration when compared with 2nd group.

Table (1): TCDD and curcumin effects on testosterone concentration (mg/dl) at day 90.

group	90 day
1st group (control)	243 a
2nd group (TCDD)	72.4 b
3rd group (TCDD and curcumin)	221.9 a

The no. of animals: 10 in each group with different letters are significantly different (*P*<0.05).

b) Semen analysis:

The sperm viability (dead and live), morphology and counting showed at table (2,3 and 4) significant decreased P<0.05 at 2nd group when compared with 3rd and control group. while 3rdgroup showed lower significant effect (P<0.05) when compared with control and higher significant effects (P<0.05) when compared with 2nd group in semen analysis content.

Table (2): TCDD and curcumin effects on sperm number % (dead and live) at day 90.

group	90 day
1st group (control)	71.9 a
2nd group (TCDD)	31 b
3rd group (TCDD and curcumin)	70.3 a

The no. of animals: 10 in each group with different letters are significantly different (*P*<0.05).

Table (3): TCDD and curcumin effects on sperm morphology at day 90.

group	90 day
1st group (control)	12.2 c
2nd group (TCDD)	34.4 a
3rd group (TCDD and curcumin)	17 b

The no. of animals: 10 in each group with different letters are significantly different (*P*<0.05).

Table (4): TCDD and curcumin effects on sperm counting at day 90.

group	90 day
1st group (control)	6616 a
2nd group (TCDD)	3121 c
3rd group (TCDD and curcumin)	5931 b

The no. of animals: 10 in each group with different letters are significantly different (P<0.05).

Pathological changes:

No important pathological changes observed in 1st group (control) were recorded (fig.1). 3rd group showed mild acute cellular swelling in seminiferous tubules characterized by degenerated sertoli cells and interstitial cells (fig. 2). The 2nd group (TCDD) administration grossly recorded passive congestion of testis, epididymis and spermatic cord with hemorrhagic spots on the tunica albuginea. Histopathological lesion showed testicular degeneration mostly nuclear pyknosis and karyorrhexis of necrotic nuclei. In some areas lacking of spermatogenic epithelium in some areas of interstitial showed lymphocytes aggregation, edema and dark basophilic necrotic cells (fig. 3 and 4). All seminiferous tubules degenerated and necrotic surrounded by mononuclear cells infiltration mostly macrophages (fig. 5).

Spermatocytic seminoma was recorded contain sloughing epithelium with pleomorphic tumor cells with highly mitotic figure, cells of tumor have large vacuolated clear cytoplasm with present of epithelioid histocytes which has eosinophilic slightly granular cytoplasm (fig. 6) elongated or ovoid nuclei with plasma cells and lymphocytes. In other section closely packed swollen polyhedral or round cells look like spermatocytes of seminiferous, some cause symmetrical testis enlargement with foamy cytoplasm (may be glycogen vacuoles) (fig. 7). Intense granulomatous reaction in the center of seminiferous tubules with thick collagenous fibers in the center of testis, congested blood vessels surrounded by mononuclear cells (fig. 8).

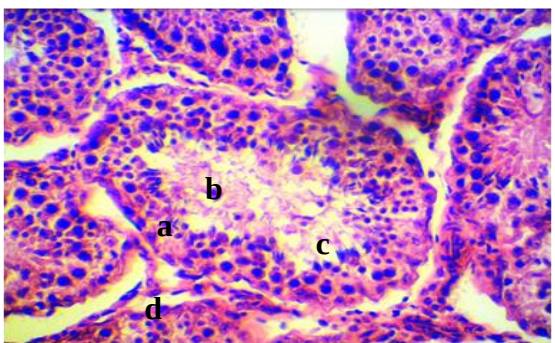
Loss of mesenchymal stroma with necrosis of sertoli cells, other sertoli cells showed malignant cuboidal cells with dark nuclei others with large vacuolated cytoplasm and completely areas hyalinized, other infiltrating sheets of eosinophilic fibrous tissue and smooth muscle cells with their relatively large nuclei (fig. 9).

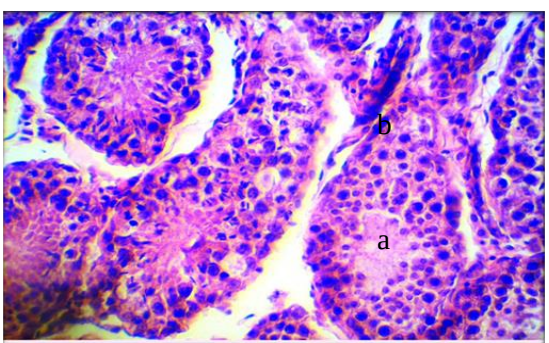
All sertoli cells and spermatid cells elongated and palisanding cells have dark nuclei with mitotic figures, some sertoli cells round or polyhedral, scanty stroma, seldom cytoplasm disarrangement of interstitial cells mostly leydig cells (fig. 10).

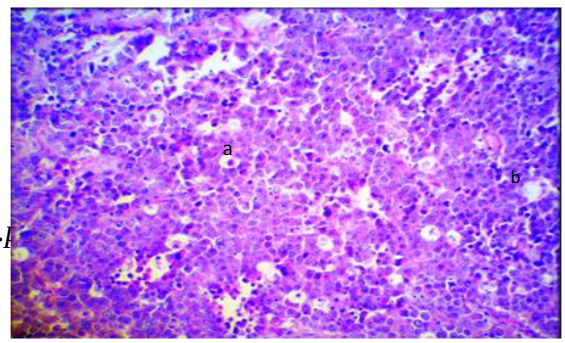
Large solid tumor masses replacing seminiferous tubules composed of polyhedral cells, leydig cells with abundant stroma (fig. 11).

Most areas of seminiferous tubules necrotic and damaged separated by thick solid bands of partially hyalinized fibrous tissue with wide areas of eosinophilic proteinous exudate (fig. 12).

The seminiferous tubules separated from each other's by thick, frim tissue and take picture of solid tumor closely packed consist of leydig and sertoli cells cancer characterized by prominent nuclei, multinucleated cells (fig. 13).



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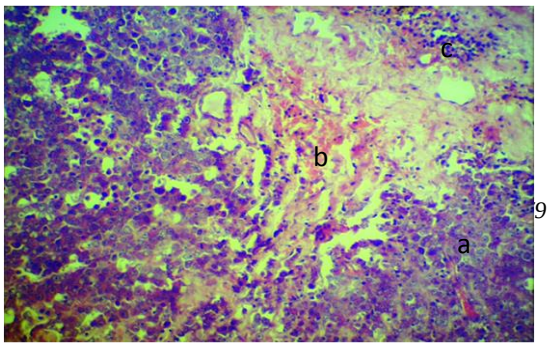
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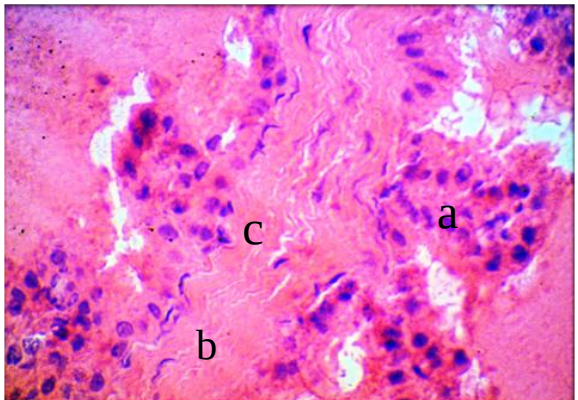
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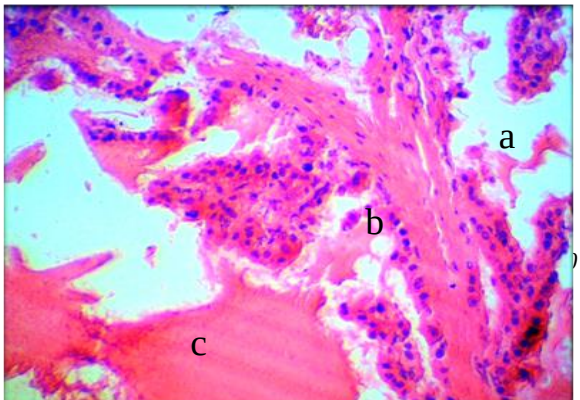
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Discussion:

Testis is a potential target organ for direct TCDD effects in adult rats which cause aryl hydrocarbon receptor (AhR) and nuclear translocator of the receptors reduction in testis and this reduction function mostly due to deformation of leydig cells and associated with severe histopathological changes and damage in testis (21,22 and 23).

About 50% lowered in sperm analysis (counting, viability and morphology) has been found in study (24 and 25) in young man in Japan after exposure to toxic effect of TCDD, the TCDD has been recorded to cause alteration sperm analysis due to direct toxic effects on sperm number production and its direct effect on testis

histology (spermatid cells and sertoli cell damage) causing inability of testis to production of sperm,

Dioxins bind to cellular protein, the aryl-hydrocarbon receptor (AhR) which regulates gene expression, whether adverse effects result from this binding depend on what biological response follow causing increased cancer incidence (26).

The TCDD is a reproductive and developmental toxin including abortion change in developing male reproductive system (27). Chronic administration of TCDD to rats in a three generation reproductive study caused significantly decreased fertility in the F1 and F2 generation but not in the F0 generation, fetal anomalies, feto toxicity and maternal toxicity (28 and 29).

The daily intake Curcumin is antioxidant and potent inducer of detoxifying and thereby prevent the toxicity induced by chemical carcinogen and used as positively effects oxidative balance and seem to be beneficial for reversing the negative effect and oxidative stress of TCDD toxicity (11).

The histological investigation showed chronic dose of TCDD cause damage of male reproductive system directly on the testis or indirect by on the endocrine regulation of testis (30).

In the current study antioxidant enzyme curcumin treatment at dose 100 mg/kg/day for 90 day significantly($P<0.05$), TCDD effects on hormonal, sperm and pathological change and potent inducers of detoxifying and thereby prevent the toxicity induced by chemical carcinogens and used as positively effects oxidative balance and seem to be beneficial for reversing the negative effect and oxidative stress of TCDD toxicity (11).

Conclusion: The histopathological examination refer to leydig& sertoli cells carcinoma (testis carcinoma).

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