CHROMIUM INHIBITS *IN VITRO* VIABILITY AND STEROIDOGENIC IN RAM LEYDIG CELLS

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(Received 4 January 2017, Accepted 16 November 2017)

Keywords: Chromium, Leydig cell, DNA.

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ABSTRACT

One of an ecological hazardous agent Chromium; Cr (VI), The present experiment *in vitro* design to examine the mechanism of Leydig cell functions of Cr (VI) in ram testis, Cr (VI) treated groups demising cell growth behavior of exponential phase was upset of feeding time to Leydig cell in a dose-dependent manner, and induced mitochondria-dependent ATP depletion and subsequently apoptosis. Cr (VI) effect may be attributed, at least in part to DNA fragmentation increase DNA tail number and tail length of COMET as compared with control group. Furthermore, the properties of cell-specific regulation of cell membrane integrity had reduced and determinant cell concentration drop an.d reflected on the testosterone concentration were decreased as concentration-dependent manner, In conclusion, our results display the Cr (VI) is cytotoxic and impairs both viability and steroidogenic functions of Leydig cells in ram testis via actually different pathway direct affecting of viability and indirect on steroidogenic activity, succeeding in testicular performance. However, the definite modes of action of harmfulness are not evidently unknown and must be rechecked and studied in a different aspect.

INTRODUCTION

Chromium, a trace element universally present in the environment and is a naturally occurring element originate in rocks, volcanic fume and earth grasses, soils containing Chromium as well as contamination of plants and animals, has been commonly recognized to cause harmful effects to humans and animals. It is commonly used in three basic industries: manufacturing, chemical compound and refractory materials which lead to environmental pollution (1).

Chief uses of hexavalent chromium compounds comprise metal plating; manufacture coloring agents, corrosion protection agents, and leather and wood preservation agents (2).

Chromium had been confirmed for its potential genotoxicity in an animal model (3) in chromium exposed human (4). It induced DNA injury by block both DNA replication and transcription. It is initiated and promotes carcinogenicity in animal models and increase the percentage prevalence of chromosomal aberrations by express sister chromatid defect, and developmental abnormal cell transformations as well as genetic mutations in mammalian cell cultures (5, 6 and 7)

Epidemiological reports, Organic and Inorganic Chromium compound exposure is an environmental problem worldwide due to the many of exposure sites that had been identified, particularly in Asia. Chromium exposure in drinking water had developed an important concern in East and Middle East area (8). Chromium had also been reported in accidental contaminant animals (9 and 10).

Exposure chromium induces a wide variety of effects, including endocrine disruption (11 and 12). Endocrine disruptions are known as any substance that can interact with hormone homeostasis and function. Substances that can play as endocrine disruptors provoked or block the function of physiological hormones and shifted an irregular response in the cells one of these is Chromium. The toxicity of Chromium as potent reproductive disorders and life jeopardy challenge has been seen in mammals. It was reported that Chromium alters gene regulation by disruption of the closely related steroid hormone synthesis functions (13, 14 and 15). However, studies pertaining to the precise mechanism of the direct effect of Chromium on ram Leydig cells viability and function are very limited.

Chromium can occur in numerous oxidation states (−2 to +6), of which the trivalent (III) and hexavalent (VI) forms are of ecological position (16). The two main hexavalent chromium forms commonly found in the environment as CrO7 − or Cr2O4− can freely pass bilayer membranes by basic anion carriers, whereas trivalent form is less transported through biological membranes. Furthermore, in the cellular and molecular events, the hexavalent Chromium endpoint is reduced to the trivalent form via the formation of series of reactive intermediates oxidize Chromium forms as pentavalent and/or tetravalent forms (17). The trivalent form complexes with intracellular macromolecules, including molecular material and cellular events, It is mutagenic capacities of chromium (18 and 19). The Chromium complexes are not

biodegradable and load an accumulative potential in the body subsequent in indirect and direct harmful effects on cellular and systematic performance (20).

“Hexavalent chromium is 100–1000 folds more hazardous toxicity than the record common trivalent complex compounds (22 and 25)”. It is typically associated with oxygen and it is an oxidizing cause generally. An accidental or experimental exposure to chromium VI has been reported to cause reproductive impairment in human and laboratory animals (21). A reduced spermatogenic cells absolute count and motility indices, as well as amplified follicle stimulating hormone (FSH) levels, were occurring in men employed in electroplating (22). Moreover, an increase in abnormal spermatozoa was observed in murine treated/ exposed to chromium (22, 23, 24 and 25).

The present study, the direct effects and harmful mechanisms of chromium, in the pentavalent form of ram Leydig cells, were tested using the culture media in vitro. In ram testis, steroidogenesis could be induced by LH in vitro or treatment with GnRH. Furthermore, was planned to assess the possible effects of different doses of Chromium on ram testicular steroidogenesis and to ascertain whether these changes are mediated the apoptosis and viability of Leydig cells.

MATERIAL AND METHODS

Animals and Ethic

Leydig cells were isolated from the testes from ten rams; males weight 28-32 k g; (9-12 months), were purchased from a commercial local market. Rams have slaughtered decapitation before dissection and the testes kept in cold Ringer solution tanks at 15 °C to use. The experimental procedures flowed with the Standard Guidelines for the Care and Use of Laboratory Animals in Research and Teaching in Iraqi Scientific Institutions IUCAC (Al-Bayati and Khamas, 2015).

Preparation of testicular organ:

Testicular tissue isolation and culture methods were carried out following Shuying *et al*. (21) with partial modification. Twenty testes from ram were used *in vitro* experiments expressive twenty replicates per treatment. Freshly removed testes were cut into pieces and placed in the dissociation buffer medium pH 7.4 and exposed to enzymatic digestion; collagenase 5 mg (type I, 213 U/mg; Thermo fisher Scientific Inc., Cat No. 17100-017, USA) in a buffered media comprise; i) M199 medium i) 2.2

g/L of HEPES, iii) Bovine serum albumin 0.1%, iv) 25 mg/l of trypsin inhibitor, v) 0.7 g/L of Sodium bicarbonate, in the water bath with shaking (80 oscillations per min) (27 and 28).

The culture medium was diluted and filtered; a nylon mesh (100 µm). The cells were then precipitated via centrifugation; 1.500 rpm for 10 min., and re-suspended in 2 ml of the culture medium mentioned above.

Purification of Leydig cells was purified by four-layer Percoll (Sigma-Aldrich, MO, USA) and (95% 1× Hank's balanced salt solution, HBSS; Thermo fisher Scientific Inc., Cat No. 14025092, USA) density gradient (21, 26, 37, and 60%) in conical tubes. The gradient solution was centrifuged at 3,000 rpm/min for 30 min at 4°C; the interface between 37% and 60% was collected and immersed in the medium to eliminate the Percoll O'Shaughnessy *et al*. (29).

The Leydig cells purity was determined by 3β-HSD histochemical technique, 1% v/v etiocholanolone was noticed to be 95 % (30), The Leydig cell viability was as evaluated by trypan blue exclusion, was superior to 85% Guoxin *et al*. (31).

Chromium treatment: Cells were seeded and allocated to 6×105 cells per well, onto 96 well, culture plates and incubated for 24 h at 37 °C with DMEM medium containing 1% Fetal Bovine Serum, in a humidified incubator and of 5% CO2. The preserved, purified cultures of Leydig cells were tested at different Chromium concentrations of cells; Na2CrO4 0, 3, 5, 10, 20 and 40 µM, the Leydig cells steroidogenic and bio-vital parameters were assayed

Cell proliferation assay:

The trypan blue assay for the viability of the cells was greater than 95% (9). Leydig cell viability was calculated as the number of viable cells divided by the total number of Leydig cells via the hemacytometer. The cells colored trypan blue, they were measured as dead cells "unavailable" cell (49).

MitoTracker probes of cell integrity

1. MitoTracker green 50 nM (Invitrogen, M7512) and Hoechst 33342 1 mM (Sigma, B2261)
2. The mixture was incubated in the Leydig cell culture media for 40 minutes.
3. The cell cultures were visualized and scanned by fluorescence microscopy "Axiovert 200 M; Carl Zeiss" (38).

MTT cytotoxicity assay:

The viability of Cell was estimated by MTT assay (48). So as to determine the cytotoxicity of Chromium on cells (5×106 cells/ml) were preserved with chromium at different concentrations for 24h. Control cells were treated with DMSO only. One 50 µl of MTT (10mg/ml) was treated for each well and the plates incubated for four hours at 38±0.5 °C, then 1ml 0.01N HCl in HEEPS was played additional cells solubilizing the cells. The absorbance was determined with the APLE PD-303UV, INDIA spectrophotometer at 570nm as test wavelength with as reference wavelength 690nm. "The absorbance was calculated as the difference between the absorbance at the reference wavelength and that as the test wavelength (49).

Percent viability = (Absorbance of treated drug of incubated cells) /(control absorbance) ×100

Lactate dehydrogenase assay

The quantity of lactate dehydrogenase (LDH) released by the Leydig cells was assayed according to the modified method of Matsuki (2000). The Leydig cell culture supernatant (100µl) was mixed with 100µl of the LDH mixture substrate (2.5mg/ ml L-lactate lithium salt, 2.5mg/ml NAD+, 100 µM MPMS, 600µM MTT, and 0.1% Triton X-100 in 0.2M Tris-HCl buffer, pH 8.2). The reaction time was carried out for 5min at 37°C and fixed by adding 0.5ml of 0.04N HCl in HEEPS. The absorbance was determined by spectrophotometer at 570nm as test wavelength and at the 655nm reference wavelength. In these assay conditions, MTT has converted into MTT formazan in proportion to LDH activity.

LDH release = (LDH blank)/(Total LDH-blank)× 100

Mitochondrial and cell membrane integrity of cells

The functional properties of membrane and mitochondria were approved with the integrity of continuous and successful integration and composed their orchestrate function. These assayed with mitotracker probe that drove according to Miyake et al., (2011).

Mitotracker probe preparation:

Mitotracker green stain stock 1 mM was diluted with a diluent mixture of DMSO 25µg CMXROS at 47µl, genital shake and used.

Culture cell staining:

The cells cultures were adjusted until reach a density 2×106 cells per ml. Mitotracker were mixed with the harvested standardized diluted cultured cells in sight media of

final concentrations; 1 µl to 5 ml cell tissue culture suspension. The staining cells were preserved in an incubator for 30 min at 38C°.the fluorescence 576 nm wavelength adjusted in fluorescence microscopy cells were check illuminated tenacity and number “red filter” emitting red fluorescent light mitochondrial activity and green for membrane integrity. The intensity of colored patches in the cells was determined and calculated with Image J software. The values referred directly to total mitochondrial mass (green) and membrane integrity and activity.

ATP concentration in cultured cells

The ATP concentration was determined in the cells in three steps as follows: (46 and 47)

The scratching cultured cells was centrifuged 300 rpm, 5 min separated then adjusted the cell concentration to 0.5×106 and stored in liquid nitrogen -196 C° Salin *et al.* (52).

Leydig cell wash: The pellet cells were thawed suspended by normal saline 1%, 5 C°, the cells were settled, separated and resuspended in three times, the separated cells.

ATP Extraction: The ATP freeing from the cells was possessed by added 0.1ml of 0.6M at 5C° perchloric acid was mixed genteelly cells for 20 min; the cells suspension was centrifuged 80000 rpm, 5 min. The cells was separated and suspended with 50µl, 3.5M of K2CO3, then centrifugation for 5 min at 8000rpm

ATP analysis: The analysis media composed: 100µl NADH-linked enzyme, as stock 1mg/ml; 20µl glucose-6-phosphate dehydrogenase, hexokinase as stock 1mg/10.5ml; 60µl glucose as stock 9mg/10.5ml; 50µl NADP-nucleotide, dinucleotide phosphate 10mg/10.5ml; 0.1 N of 800µl TRAP buffer pH 7.6.

The spectroscopic analysis of ATP concentration was assessed at 340 nm the value calibrated with a standard curve of ATP previously prepared for multi dilutions, Mitra *et al*., (51).

DNA Quantity “COMT assay”

The comet tail and incidence was examined by fluorescent microscopy “close related to the head like emittances reflection DNA damage”. Concurrent comet slides sets contain slides negative only adjuvants and positive control “self-control”.

The scoring COMETs data was expressed as comets number/slide/culture, approaches for the quantities of DNA concentration in normal and damaged DNA criteria.

COMET procedure

The prepared agarose slides were dipped in a lysing media for 12 hours contain; NaCl 2.5 M, Na2EDTA 100 mM, Tris pH 10 +10% DMSO 10 mM, Triton X-100 1%

Slides were dipped in 10 mM dithiothreitol for 20 min in within lysis media. Transfer the slides gel electrophoresis buffer, alkalization COMT assayed for denaturation of DNA, 10 min at 4 C˚ in alkaline electrophoresis buffer: NaOH 300 mM, Na 2EDTA 1mM, and HCl pH Modifier amounts

Electrophoresis migration was done at the following tenets; 27 V: 0.8 V/cm by 300 mA at 4 C˚ for 5 min.

The slides were incubated in 0.3 M Sodium acetate-ethanol base incubated 30 min. and dried in 99% ethanol 1 hours and 70% ethanol for 5 min. Air-dried slides (25±2C˚), staining slides by 12 mg/ml ethidium bromide; fluorescent microscope detecting slides with X 200, and measured by "Image J" program. Scoring the DNA/100 cells and quantifies tail length percentage of damaged DNA.

The comet concept calculation based on migration length and the migrated DNA fragment percentage, Tail ═ Tail length × DNA in the tail (46 and 47)

Determination of steroidogenic activity in Chromium containing media

1. The concentration of Leydig cells 10×106 in 24 whole plates with culture medium containing Chromium and charcoal-stripped fetal bovine serum 10% v/v as well as penicillin-streptomycin 1%, incubated in O2: CO2 (95:5%), 38 ˚C, 24 hours.

2. hCG was added in culture media and incubated for 2 hours in the same conditions, the cells were harvested and centrifugation at 2300 g for 15 minutes and separated supernatant.

3. The supernatant was incubated in 80 C˚ for 5 minutes and centrifugation at 2300 g for 15 minutes, kept for testosterone estimation by RIA. (46) .

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from Leydig Cell Culture Medium The supernatant Leydig cell culture medium was examined by using Testosterone RIA. Testosterone measurement manner was carried out at the RIA lab-Baghdad.

Statistical Analysis

The real examination of the control and treated assembling were subjected to examination of analysis of variance (ANOVA) two-way analyses. A probability of $p<0.05$ was required to indicate a significant difference. Each group comprised, in any event, six repeats. LSD test was used for comparison between groups. A correlation between responses was made (16). The best-fitted curve was managed by the following equation y = bm * x.

RESULTS

In this study analyzed the effects of chromium on ram Leydig cell testis in vitro under influence of LH. Testicular Leydig cells of the control culture contained normal viable cells after 2 days of culture. However, culture Leydig cells treated with 100 µM chromium exhibit cell death. Moreover, extensive cell death was observed in groups treated with 200 µM chromium compared with those treated with chromium lower concentration (Fig. 1C and D). There was no observed change in a structure such as a cell death, degeneration, or hypertrophy in testis exposed to 0.1–10 µM of chromium.

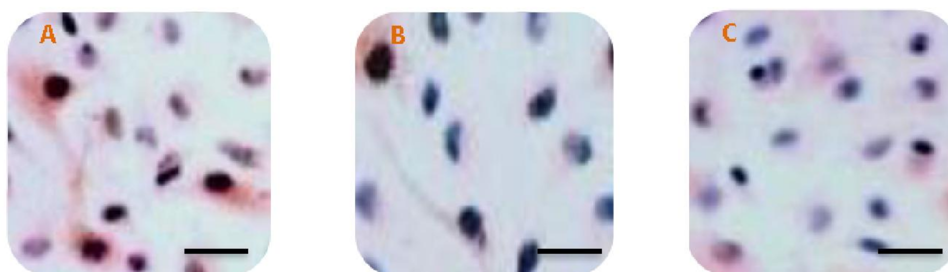

Figure 1. Representative light micrographs of ram testicular Leydig cells cultured for 2 days (A) Control and (B) low chromium concentration () Normal morphology were observed with many Leydig cells. (C) 100 µM chromium. Some dead germ cells were observed (D) and 200 µM chromium, A notable increase in the number of dead Leydig cells was observed with amplification of Leydig cell volume. Bars, 20 µm

The influence of chromium on the progression of Leydig cells examined by 5-bromo-2-deoxyuridine (BrdU) incorporation in Leydig cells is shown in Figs 2A–G and 3. After treatment with chromium, the number of proliferating Leydig cells increased

after 2 days 100 μM (Figs 2A and B and 3A) and 200 μM of culture (Fig. 3B). However, the addition of arsenic significantly inhibited LH-induced Leydig cell proliferation in a dose -dependent manner (Figs 2C–F and 3A and B). In cultures treated with 100 μM chromium and 200 μM chromium, not even a single BrdU-positive germ cell was detected due to extensive cell death (Figs 2G and 3B). Treatment with chromium alone at lower doses (0.1–10 μM) seemed to have no effect on Leydig cell proliferation both cultures respectively. However, at the highest dose of chromium (200 μM) alone, the number of BrdU-positive germ cell significantly decreased (data not shown).

The parameters viability, cell membrane, mitochondrial integrity and ATP concentration, testosterone concentration as well as apoptotic percent were significant ($p<0.05$) decrease as dose manner dependent figure (2, 4, 5, 7, 8 and 11). On the other hand, the lactate dehydrogenase and DNA tail length were significant ($p<0.05$) increase as dose dependent figure (9 and 10).

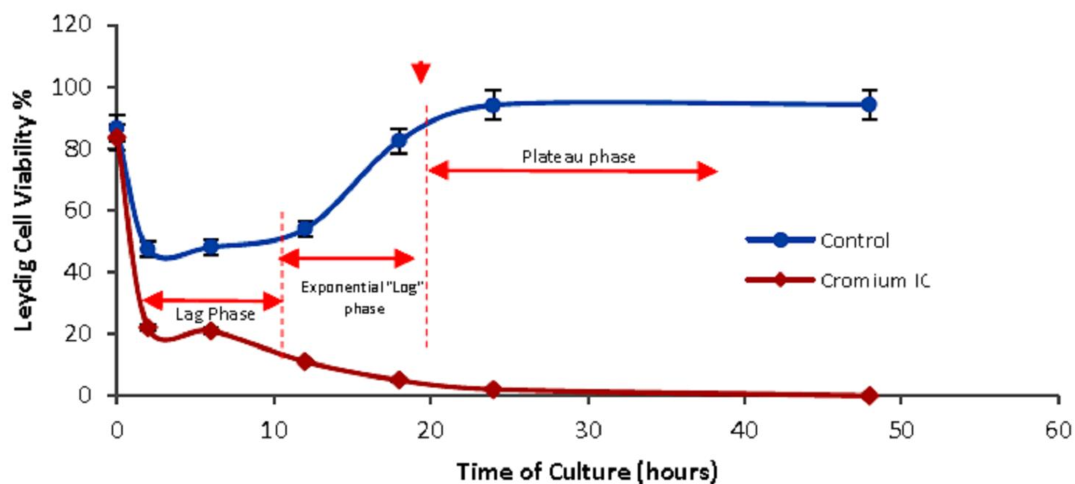

Figure 2. Growth curve and culture maintenance, the plot of Leydig cell viability concentration% versus time hours from subculture, showing in control the lag phase 12.056±0.681, exponential phase 12.703± 1.35, and plateau started 24.833 and indicating times which subculture and feeding. The Chromium treated showed behavior growth curve drop maintenance value as well as lost feeding pattern. n=10

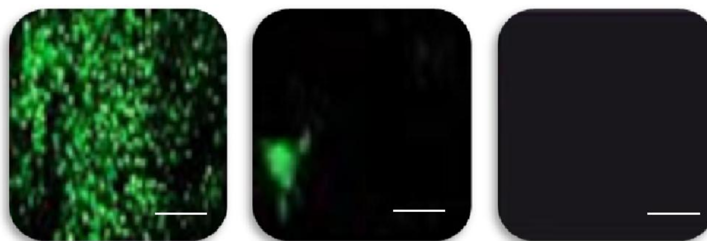

Figure 3. Leydig positive cells are MitoTracker positive. Leydig cells were MitoTracker and Hoechst Expression of Viable and active mitochondria was visualized by fluorescence green tendency. Micrographs shown were presented control (a), low (b) and high concentration (c) by fluorescence microscopy. (Scale bar in = 20 µm).

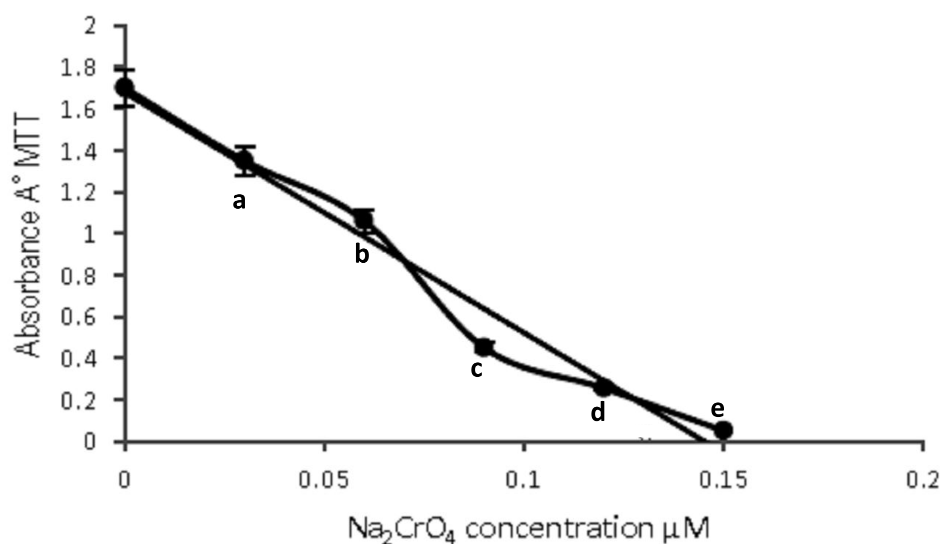

Figure 4. Log dose-response relationship of Cr (VI) abolished viable cells value (MTT) in ram Leydig cell through cumulative cell proliferation. The letters denoted difference $p<0.05$ between the groups n =10 samples of Leydig cells isolated

Data are presented mean ± SE

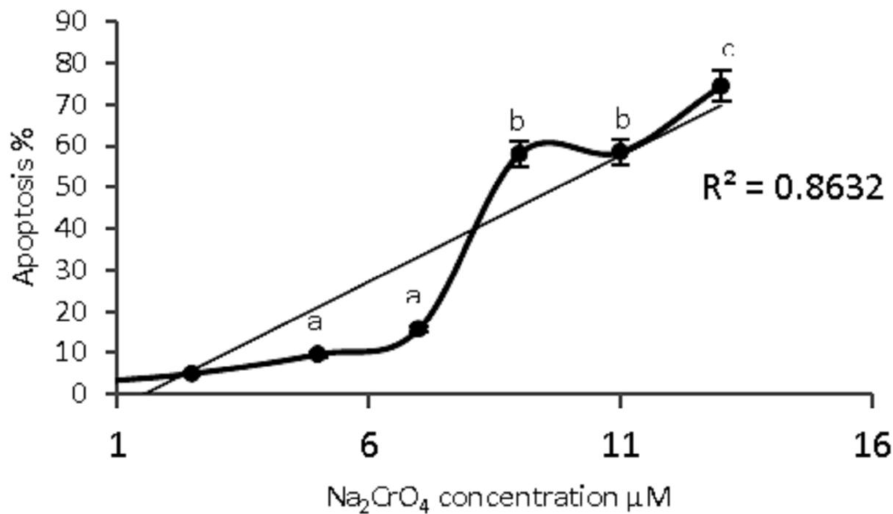

Figure 5. Log dose-response relationship of Cr (VI) -promote apoptosis in ram Leydig cell through cumulative cell proliferation. Leydig cells were harvested at 10 cell culture passage and treated with 0 to 13 µM Na2CrO4 for 18 hours. Harvested Leydig cell trials were investigated for a present fraction of apoptotic cells by Phosphatidylserine translocation. The fractions of apoptosis in Leydig cell denote the means of the 20 culture trial of Leydig cell culture passage numbers.

Data are presented mean ± SE

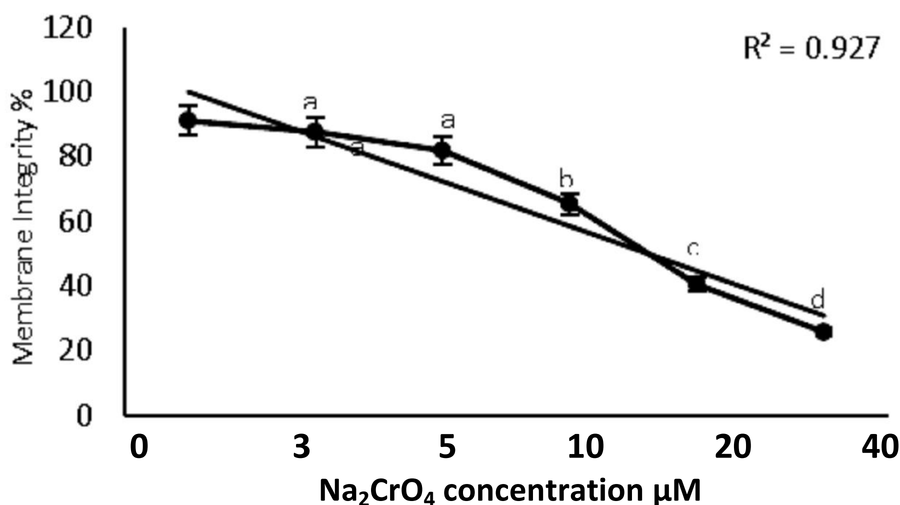

Figure 6. Log dose-response relationship of Cr (VI) –induced reduction of membrane integrity in ram Leydig cell through cell proliferation, Leydig The fractions of apoptosis in Leydig cell denote the means of the 10 culture trial of Leydig cell

culture passage numbers. The capital letters denoted difference $p<0.05$ between groups n =10 samples of Leydig cells isolate ,Data are presented mean ± SE

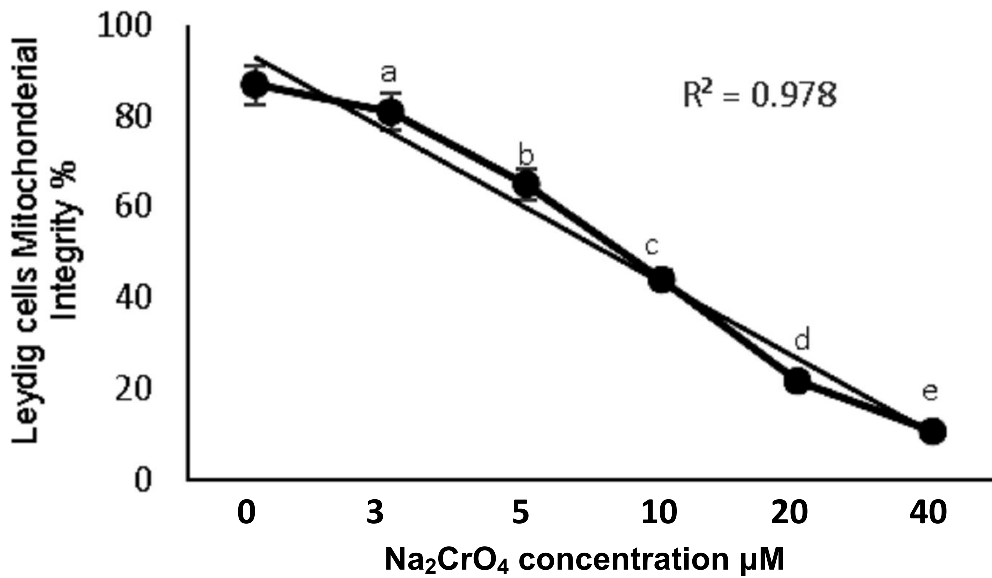

Figure 7. The Cr (VI) effect on mitochondrial integrity Leydig cell in ram Leydig cells of In vitro culture. The capital letters denoted difference $p<0.05$ between groups, n =10 samples of Leydig cells isolate.
Data are presented mean ± SE

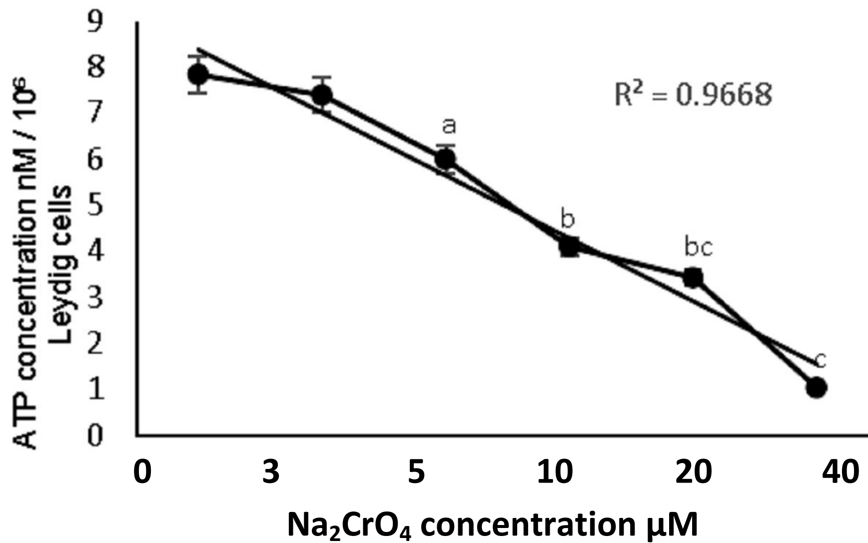

Figure 8. The Cr (VI) effect on ATP concentration of Leydig cell in ram Leydig cells of In vitro culture. The capital letters denoted difference $p<0.05$ between groups. n =10 samples of Leydig cells isolate
Data are presented mean ± SE

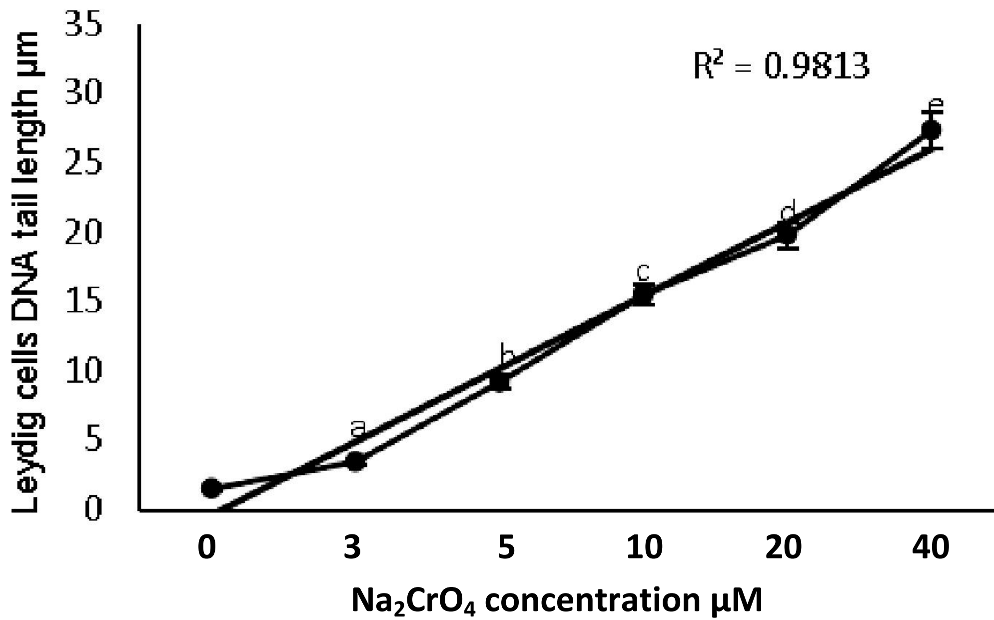

Figure 9. The Cr (VI) effect on DNA tail length Leydig cell in ram Leydig cells of In vitro culture. The capital letters denoted difference $p<0.05$ between groups n =10 samples of Leydig cells isolate

Data are presented mean ± SE

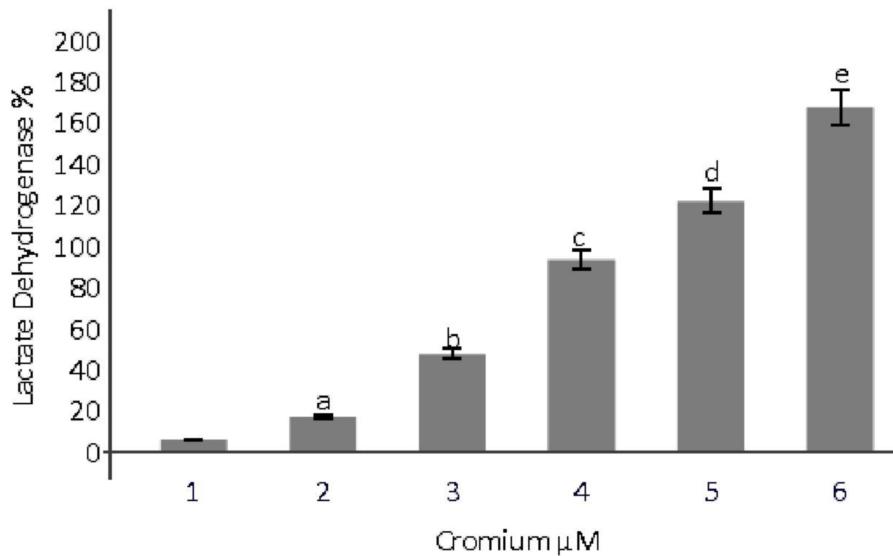

Figure 10. The Chromium effect on lactate dehydrogenase of Buck Leydig cells of In vitro culture The letters denoted difference $p<0.05$ between groups n =10 samples of Leydig cells isolate

Data are presented mean ± SE.

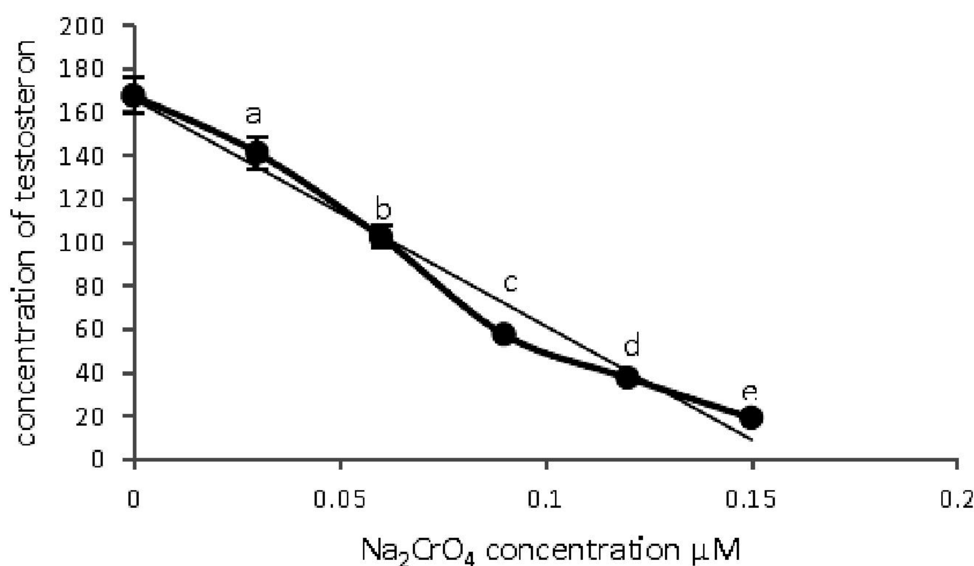

Figure 11. The Cr (VI) effect on testosterone Leydig cell in ram Leydig cells of In vitro culture. The capital letters denoted difference $p<0.05$ between groups n =10 samples of Leydig cells isolate

Data are presented mean ± SE

DISCUSSION

The experiment aimed to determine *in vitro* viability and steroidogenic effects of Cr hexavalent on Leydig cells of the ram, which have critical roles in orchestrating some testicular activities. The experimental design was explored Cr hexavalent effect on apoptosis-related signaling mechanisms in the proliferation of cells. For this purpose, cells were treated with Cr(VI) at doses of 0, 3.125, 6.25, 12.5, 25, or 50 µM for 24 h; and then check viability, steroidogenic activity, apoptosis as well as both DNA and ATP.

To the dominant of the idea in this study is the first to report that the dose-dependent cytotoxic effects of Cr (VI) exposure in Leydig cells are mediated through apoptosis. Since Leydig cells had an important role in the progressive development of spermatogenesis, impairment and/or damage to these cells has unwanted effects on the normal fertile sperm cells. The study examined whether DNA figure 9 and

mitochondrial-ATP system dysfunction figure (7 and 8) associated with exposure to Cr (VI) decreased the Leydig cells viability figure (2, 3 and 4) and increase apoptosis, which was presumably reflected their effect on the production of testosterone figure (11), which was upheld up to 24 h. In contrast, 24 h exposure to Cr (VI) decreased testosterone in a dose-dependent manner.

Degeneration and diminished of Leydig cell concentration in treated groups has been attributed to the intoxication of hexavalent Chromium through attendant presumably particulate diminished nucleic acid bases and metabolic processes as apparent from narrowing of the Leydig cell diameter Figure (1), these were reflected to reduce steroidogenic yield figure (6). From results, findings, it may presumably indicate that harmful concentrations of hexavalent Cr had hampered the Leydig cells function causing impairment of the normal steroid biosynthesis functioning of the cultured cells (33).

The other words, on the conventional form by reduction of Cr6 to Cr3 Intracellularly led to promote the formation of complex “DNA-phosphate-based” adducts that reason a variety of direct genomic impairments and lost their belonging to testosterone (34).

The genetic deterioration which sequentially downregulates the “Trp53 signaling pathway” these crafted a presented programmable apoptosis due to Leydig cell cycle arrest (35). Furthermore, Cr treated caused the decrease of nucleotide excision repair mechanism, which develops cytotoxicity (36).

The investigation of the apoptosis encouraged by hexavalent Cr in the somatic cell isolated; Leydig cell culture sheep testis cell system consisting of controlling serpentine pathway revealed to trp53 is essential for harmful Chromium trigger expression of the BH_3 proteins. That may be explained gradual increase apoptosis as sequences of series chromium concentrations figure (5).

Otherwise, Trp53 trans motivates a sequence of pro-apoptotic proteins from the BCL2 family;” BAX, PUMA, and NOXA” (37), these p53 reported controlling factor of permeabilisation in mitochondrial membranes according to this fact act as initiator release apoptogenic reasons from the mitochondrial intermembrane space, as a transcription-independent manner directly extend DNA damage promotion apoptosis considered intrinsic pathway including release of cytochrome c, That propose the

mitochondrial death pathway modulate apoptosis is presumably triggered by Cr, (37). Cr fact induces p53 protein growth arrest that may be reduced, total count as an endpoint and dramatically indirect upset the testosterone concentration figure (11).

Furthermore, Chromium hexavalent compounds are unreactive with bases of nucleic acids of DNA in normal functional conditions of cells. But in produce defect presumably due to the fact the Cr^{+3} transforms to a sequence of reduction stages in cells, until thermodynamic stability Cr^{+3}. A molecular event of reduction was reacted non-enzymatic process, whereas, the reduction formation was facilitated through the electron transfer system from both ascorbate as well as thiols “glutathione and cysteine” (40). The harmful chromium process exerts during the reduction progression events, and promotion, organic radical species with certain Cr^{+5} and Cr^{+4}, depends mainly on the reducing species (39).

The resultant Chromium induces DNA defect in the Leydig cells *in vitro* may be revealed a major influence of the intracellular reduction activity on the physiological state consequences of the resultant DNA lesions. That may be presumably suffered from triple modifications to DNA comprise i. Chromium (III) - DNA adducts, ii. DNA–protein and DNA–DNA inter strand crosslinks, iii. DNA breaks along with several oxidative DNA-base modifications. These adducts are formed essentially at phosphate groups, but the consequent particularly creation of chelates including the phosphate group and the guanine (N7-position) had been suggested (41).

Based on this outcome, figure 9 of DNA, a classical may be created to an indication the chromium treatment provokes the “selective outgrowth of mismatch-repair-deficient clones” with high rates of unplanned coding nucleic bases of genes, and created genomic instability (42 and 43). This indicated to presumably lead to the endpoint of the upset of steroidogenic activity as testosterone link detail, figure11.

The Chromium harmful effects may be exerted the second line through cellular reductants, potentially disruption intermediates; facilitated formation; oxygen, sulfur, and chromium radicals are generated (44). In a cell-free system, chromium (VI) high affinity with glutathione (Fenton-type reactions form hydroxyl) is formed Cr^{+5} as well as thiyl radicals under ascorbate (45).

This proves that mitochondrial activity reduction figure (6) and subsequent ATP depletion figure (7) play a crucial role in Cr hexavalent induced cytotoxicity. The

study showed that maybe the transcriptional expression of antioxidant enzymes that play a significant role in scavenging free radicals, including Cat, Sod1, Sod2, Gpx1, and Gsta424–29 decreased with increasing doses of Cr (VI) (25). Thus, the oxidative stress in the Leydig cells may be proposed after exposure to Cr (VI) appears to be due to poor scavenging of free radicals by the antioxidant enzymes through the direct deleterious effect of Cr. These results are supported by those of previous reports demonstrating that Cr (VI)-induced oxidative stress via the suppression of antioxidant enzymes plays a major role in male infertility (44). The malformation of Gsta1 seen with doses of Cr (VI) in somatic cells might protect the cells against Cr (VI) -induced oxidative stress.

Cell viability depends on the balance between survival and pro-apoptotic signaling, regulated by the AKT1, MAPK, and Trp53

Pathways (32 and 33) AKT1 and ERK1/2 signaling pathways are associated with cell proliferation and survival (34,35), while ROS produced by several toxicants, including Cr (VI), serve as second messengers to activate pro-apoptotic kinases, such as JNK1/2 and P387, (36–43).

Cr (VI) -induced DNA damage and oxidative stress also promoted the activation of P53 leading to intrinsic apoptosis33, 44–47. We showed that, in male somatic cells, exposure to Cr (VI) increased the phosphorylation of JNK1/2 and P38 induced intrinsic apoptosis in Leydig cells in a dose-dependent manner.

Loss of cell viability observed at higher doses of Cr (VI), involves the apoptosis pathways and impairment of the GDNF signaling pathway and decrease testosterone

A number of researchers have shown that impairment of steroidogenesis by environmental toxicants does not require the inhibition of all the steroidogenic enzyme genes (2). In the present study, therefore, Cr (VI) impaired steroidogenesis, at least to some extent, may be due to inhibiting Cyp11a1 and Hsd3b1 in TM3 cells, Cr (VI) also decreased the expression of Cyp19a1, which is required for the conversion of androgens into estrogens.

The Bcl-xl protein (53) inhibits apoptosis in a variety of cell systems. Following Cr (VI) administration, Bcl-xl levels are transiently reduced in interstitial cells. This decrease in Bcl-xl is referred to including the start of apoptosis within the Leydig cells

(5).

However, since Leydig cells are a terminally differentiated cell type, the lack of p53 would seem understandable and cell cycle arrest unnecessary since the Leydig cells are in G_0.

Study conclusion would suggest that the Leydig cell possesses counted another mechanism for the effect of Cr (VI) that modified regulation of Bcl-xl expression which functions to maintain a proper threshold for apoptosis and to limit the severity and duration of another death promoter.

Apoptosis is a process in the cells, which is engaged in promote to specific signals (55). These categorized biochemical biomarkers for apoptosis is the activation of endonucleases, the study indicated previously that internucleosomal DNA fragmentation can be induced in Leydig cells by *in vitro* treatment with Cr (VI) (54).

Following Cr (VI) treatment few apoptotic cells were seen at 6 h, but there were large increases at 12 h up to 24 h. This increase in apoptosis precedes the decrease in the 3βHSD-positive Leydig cell numbers by about 6 h. These observations are in agreement with previous studies in which apoptotic changes in cell morphology were noted, followed by the phagocytosis of the dying cell by neighboring macrophages (55).

The study concluded the chromium impaired the sterodoginic activity and deregulation of Leydig cell development, via direct affecting on DNA, mitochondrial activity and structural injurious, promote cell death.

ACKNOWLEDGMENT

I acknowledge the training and ethical support to Prof. Dr. Wael Khamas, (College of Veterinary Medicine; Western University, California, Pomona) for the Leydig cells isolation protocol and his valuable comments with advice, and Andrea Wournell; (molecular Biology, Lab; Western University, California, Pomona) for training on testicular cell isolation

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عد الكروم احد المواد المؤذية في البيئة: الكروم السداسي في التجربه الحالية صممت لاختبار الية عمل الكروم في وظيفة خلايا ليدك لخصى الاكباش في الزجاج. مجاميع المعالجه بالكروم السداسي ثبطت صفات النمو في الخلايا لطور التعدد الاسي محدثا تتواطؤ في وقت التعزيز لخلايا ليدك في الطريقة المعتمده على الجرعه. ومحدثا استنفاذا الادنوسين ثلاثي الفوسفيت المعتمد على المتقدرات ومن ثم موت الخلية المبرمج. تأثير الكروم السداسي يعزى في الاقل على زيادة حدوث تشظي الدنا بدلالة معايير الكومت بزيادة كلا اعداد التذيل وطوله مقارنة مع مجموعة السيطره. الى اكثر من ذلك اختزلت مميزات المنظمه للسلامة غشاءء الخلية وعدد الخلايا مما انعكس على تركيز هورمون الشحمون الخصوي متزامنا مع الجرع. ومن النتائج استنتج الكروم السداسي مسم للخلايا ومبطلا لحيويتها وفعاليتها الوظيفية لانتاج الستيرويد في خصية الكبش مسارات مباشرة في الحيوية وغير مباشره في فعالية الستيرويدية ومن ثم الأنجاز الخلوي. ومع ذلك ان الانماط في احداث الضرر ليست من الواضحة معروفة وتحتاج الى فحصها ودراستها في جوانب مختلفة.

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