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Research Article

Histological, biochemical and chromosomal aberrations of pituitary gland induced by acrylamide in male rats

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Abstract

This study aimed to evaluate the cytotoxic effects of Acrylamide (ACR) on the pituitary gland and bone marrow of male rats. A total of 40 male rats were used and randomly divided into four groups, each including 12 rats. The first and second groups were considered as control and were kept for 6 and 8 weeks. The third group was administrated orally with Acrylamide at two doses/week as 4mg/kg.b.w for 6 weeks. The fourth group was administrated orally with Acrylamide at two doses/week of 4mg/kg.b.w for 8 weeks. After the treatment period, the rats were sacrificed, then their pituitary glands were removed and processed for histological examinations. Histological examination showed degeneration, congestion of the blood vessels, deposition of collagen fibers, heavy infiltration of inflammatory cells, and having pitucyte embedded with collagen. After 8 weeks post-treatment, the results showed pitucytes with vaculated cytoplasm, necrosis with degenerated pitucytes, irregular distribution of pitucytes nuclei and hemorrhage. Pituitary sections stained with PAS stain in control rats showed the homogenous distribution of the mucopoly saccharides and all cells within anterior and posterior regions. While sections of rats after 8 weeks post-treatment showed the homogenous distribution of the mucopoly saccharides in the posterior and moderate in the anterior regions. The results of chromosomal aberration in male rats' bone marrow cells treated with 4 mg/kg.bw after 8 weeks showed dicentric chromosomes, fragment centromeric separation, and ring chromosomes. The results showed a significant increase in serum T4 and a decrease in serum TSH level of groups exposed to acrylamide in both 6 and 8 weeks periods compared with control groups.

Keywords: Acrylamide, Pituitary gland, TSH, T4, Chromosomes aberration.

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Introduction

Acrylamide (ACR) is produced in carbohydrate-rich foods during cooking at high temperatures and is flexible because of its small and hydrophilic structure and circulates easily in the body (Yener et al. 2016). ACR is neurotoxicity forming in some foods at high-temperature cooking processes, like frying, roasting and baking (Pedreschiet 2014). Acrylamide is commonly used in industries, wastewater treatment, adhesives and laboratory gels (Nordin-Andersson et al. 2003). ACR was detected in high concentrations in common heated starch foodstuffs like potato products and bread (Konings et al. 2003). It has been designated as a "probable carcinogen" by the International Agency for Research on Cancer (Lyon 1994). Matose et al. (2019) showed the metabolism of ACR distributed in different body tissues such as the liver, heart, kidneys, brain, and breast. It is harmful to reproduction, and in the liver, it converts into glycidamide that damages DNA (Maan et al. 2020). In male rats treated with ACR, it stimulates an

immune response (Khalil et al. 2014; Mahmood et al. 2016).

ACR molecules are tiny and hydrophilic, which allows them to transport in every organ and of the body (Friedman 2003). It is ingested and then absorbed into the circulation, where it binds with DNA, neurons, hemoglobin, and enzymes before being transported to various organs (Baum et al. 2008). ACR may be acquired in three ways, the most common and significant of which is from the diet (Brisson et al. 2014). The hazard of ACR is a comfort to its vital transformations to more reaction of molecules that primary cellular effect, and caused pathogenic pathway is the oxidative biotransformation of ACR by cytochrome P450 2E1 (CYP2E1) (Hammad et al. 2013). ACR can stimulate damage at the gene and chromosome levels. It has an active attraction to the proteins of the sperm (Dearfield et al. 1995). But a number of no genotoxic mechanisms like endocrine effect and oxidative stress have been shown to cause several of the tumorigenicity of acrylamide in rats (Park et al. 2002). Somewhat chemicals can affect the endocrine system through interaction by particular locations, leading to alterations of physiological functions that are similar to the effect of ACR on the endocrine gland, such as cadmium chloride. Cd Exposure is accompanied by the activity of the endocrine system in both genders in rats (Borgeest et al. 2002). Mohamed et al. (2015) showed that exposure to cadmium chloride causes a reduction in thyroid hormone levels in adult rats. Based on the above-mentioned background, this study aimed to evaluate the cytotoxic effects of Acrylamide (ACR) on the pituitary gland and bone marrow of male rats.

Material and Methods

Acrylamide monomer with 99% purity was obtained from the chemistry laboratory at the faculty of science. It was melted in double distilled water to obtain a solution of acrylamide monomer and then was given at dose levels below LD50 and oral LD50 values in rats ranging from 124 to 565mg kg/bw (Karimi 2014). Forty-eight adult male rats with a body weight of 150-200 g were obtained from animal house, College of veterinary medicine, Bagdad University. Rats were kept in metal cages during the experiment under healthy circumstances. The animals were given sterile and clean water and food and kept under 12h light/12h dark photoperiod in an air conditioner animal house.

Rats were separated into four groups, each including 12 rats as follows: The first group (G1) was given distilled water and used as the control group for a 6-week experimental period. The second group (G2) was kept as a control for 8 weeks, the third group (G3) was administered acrylamide with two doses/ week of 4 mg/kg b.w diluted in 10ml distilled water orally for 6 weeks, and the fourth group (G4) was administered with acrylamide as two doses/week of 4 mg/kg b.w diluted in 10 ml distilled water and then given orally for 8 weeks.

The pituitary glands of specimens were removed from the sacrificed rats in all experimental groups at the end of the experimental period for histological examination. In addition, bone marrow samples were collected from both femurs of rats of all groups and used to study chromosomes for 8 weeks periods. At the post of treatment, the animals were sacrificed and then the blood samples were obtained from the heart under diethyl ether anesthesia. The blood was placed in glass tubes free of anticoagulant to obtain the serum. The tube was left at room temperature for the blood clot in order to isolate the serum. Separating of the serum was done by centrifugation at 3000rpm for 10 minutes, removing the serum using a pipette, then transferred into marked plastic tubes and kept at -20C until analysis.

Histological preparation: The pituitary gland was fixed in 10% formalin buffer solution for 48 hrs, and cut into proper sizes, dehydrated, cleared, embedded in paraffin wax, sectioned at 5-6μ thickness, and finally stained with the hematoxyline and eosin stain. The histological sections were examined using a light

Table 1. Effect acrylamide on body weight in male rats ((M±SD).

Group	Initial Body Weight of Male Rats(g)	Final Body wight of Male Rats (g)	Difference between initial &final weight
Control (6 weeks)	211.58±10.57	269.33±14.73	57.75±20.74[a]
Treated with ACR (6 weeks)	219.33±13.131	208.33±10.941	11.00±16.125[c]
Control (8 weeks)	212.08±11.373	272.17±16.678	60.08±15.347[a]
Treated with ACR (8 weeks)	205.83±10.624	202.50±14.222	-3.33±16.002[c]

Different letters indicate significant differences among groups at ($P \leq 0.05$)

Table 2. Effect of ACR on pituitary gland weight in male rats (M±SD).

Group	Pituitary gland weight
Control (6 weeks)	1.7433±0.4858[a]
Treated with ACR (6 weeks)	0.3583±0.0721[b]
Control (8 weeks)	1.5142±0.5610[a]
Treated with ACR (8 weeks)	0.1850±0.0385[b]

Different letters indicate significant differences among groups at ($P \leq 0.05$)

microscope (Luna1968).

Blood tests: Blood from all animals was put in tubes containing EDTA and serum was separated after centrifugation at 3000 rpm for 15 min at 4℃. Plasma was used for measuring the hormones of tetraiodothyronine (T4) and Thyroid-stimulating hormone (TSH) levels.

Hormonal analysis: The levels of TSH and T4 were assayed using commercial kits Boditech Med (Korea).

Chromosomes study: Cytogenetic analysis was performed using the bone marrow cells according to Jackson (2005), with some modifications. Animals were sacrificed by cervical dislocation. Animals were intraperitoneally injected with colchicine after 1.5 hrs. After the last dose. Both femora were dissected and cleaned of any adhering muscle. Bone marrow cells were collected from both femora by flushing in warm KCl (0.075 M, at 37°C) and incubated at 37°C for 25min. After incubation, the material was centrifuged at 2000rpm for 5min. The supernatant was poured and the cell pellet was fixed in acetic acid: methanol, 1:3, v/v. Centrifugation and fixation were repeated twice for 30 min. Cells were then resuspended in fixative, dropped onto chilled slides, and left to dry. The slides were stained in 2% Giemsa stain and washed in distilled water to remove excess stain.

Statistical analysis: Analysis of variance (ANOVA) was used in the SPSS, after which a modified Revised least significant difference R.L.S.D to compare the treatment and the control means.

Results

The results revealed no significant differences ($P<0.05$) between groups and control in all experimental periods (Table 1). The final body weight showed a significant decrease ($P \leq 0.05$) in groups exposed to acrylamide for 6 and 8 weeks. The results also showed a significant decrease ($P<0.05$) in pituitary gland weight of the treated group in 6 and 8 weeks (Table 2). There was no significant difference (P 0.05) in pituitary weight between control groups at 6 and 8 weeks. The results showed a significant increase ($P \leq 0.05$) in T4 of groups exposed to acrylamide at 6 and 8 weeks, and a significant decrease in TSH level of the treated group with ACR in both 6 and 8 weeks was also observed (Table 3)

Histological study: The histological examinations of the pituitary sections of control rats showed pituitary gland consists of the anterior lobe, revealing acidophils cells having a dark red color, and the basophils cells that had a dark color. The posterior

Table 3. Effect of ACR on serum hormone level of TSH and T4 in male rats (M±SD).

Groups	TSH /mg(µg/mg)	T4 /mg(µg/dl)
Control (6 weeks)	11.086±1.837[a]	9.430±1.472[c]
Treated with ACR (6 weeks)	2.457±1.317[b]	72.885±7.627[a]
Control (8 weeks)	10.101±2.083[a]	9.113±1.189[c]
Treated with ACR (8 weeks)	1.934±1.214[b]	62.894±6.596[b]

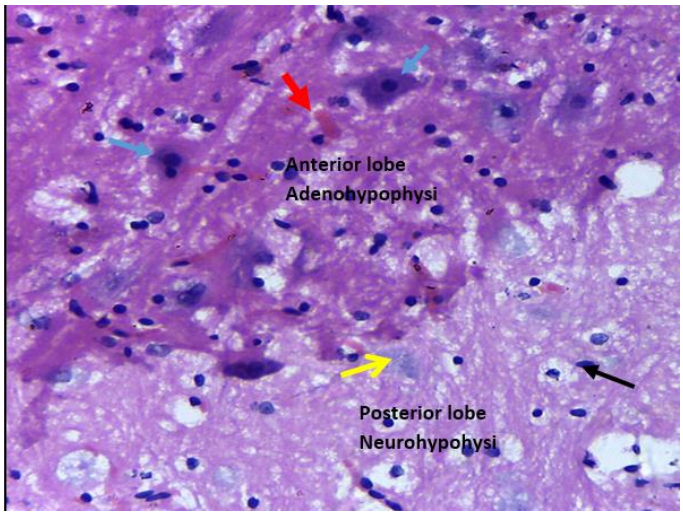

Fig.1. Cross section of Pituitary gland rats of control shows normal structure, consists of Anterior lobe, showed Acidophil cell (red dart), Basophil cells (blue dart) and Posterior lobe, showed Pituicyte cell (black dart), and herring bodies (yellow dart), H&E. 400X.

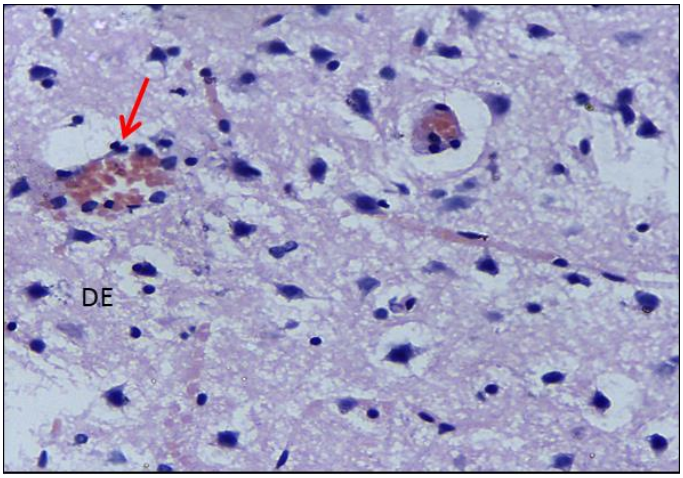

Fig.2. Cross section of Posterior Pituitary gland of rats after 6 weeks from treatment shows degeneration (DE) and congested of blood vessels (blue dart), H&E, 400X.

lobe showed a major cell type as a glial or supporting cell known as pituicyte and herring bodies. These are storage sites of neurosecretory material of the pars nervosa neurons (Fig. 1). Observation in posterior pituitary sections in rats after 6 weeks post-treatment showed degeneration and congestion of the blood vessels, deposition of collagen fibers and heavy infiltration of inflammatory cells, and having pitucyte embedded with collagen (Figs. 2, 3). The results also showed in posterior pituitary sections of rats after 8 weeks post-treatment showed, pitucytes with vacuolated cytoplasm and necrosis with degenerated pitucytes and irregular distribution of the pitucytes nuclei, vesicles with colloid (VC),

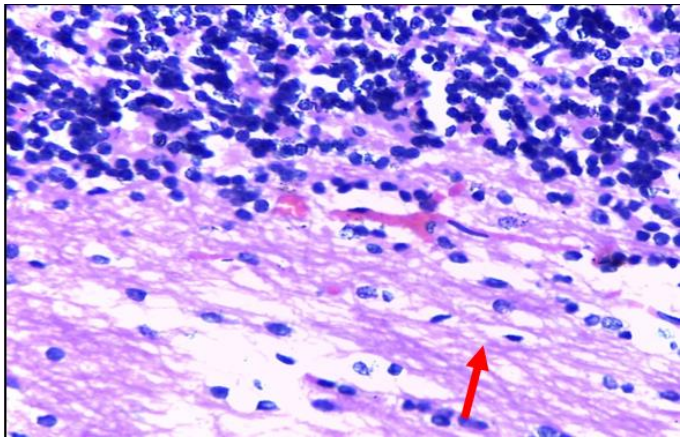

Fig.3. Cross section of Posterior and anterior Pituitary gland of rats after 6 weeks post treatment shows deposition of collagen fibers (blue dart), heavy infiltration of inflammatory cells (red dart), and pitucytes (black dart) embedded within collagen fibers. H&E, 400X.

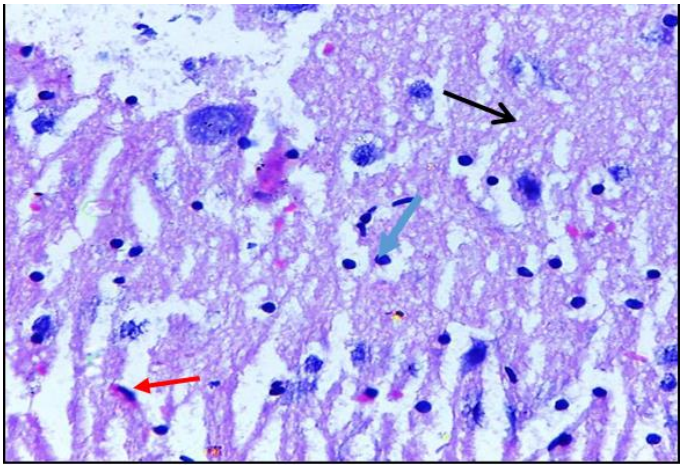

Fig.4. Cross section of Posterior gland of rats after 8 weeks post treatment shows Pitucytes with vaculated cytoplasm (blue dart), necrosis with degenerated pitucytes (black dart), and irregular distribution of pytucytes nuclei (red dart), H&E, 400X.

hemorrhage inflammation of inflammatory cells and a decrease in the pitucytes, sever congestion and pitucytes embedded within connective tissue, and normal capillaries (Figs 4, 5, 6).

In pituitary sections stained with PAS in control rats, findings showed a homogenous distribution of the mucopoly saccharides and all cells within anterior and posterior regions (Fig. 7). The sections of rats after 8 weeks post-treatment showed the homogenous distribution of the mucopoly saccharides in the posterior and moderate in the anterior region, weak reaction of the mucopoly saccharide and loss of connective tissue, and congestion of blood vessels (Fig. 8).

Chromosomal aberration: The chromosomes of bone marrow in control male rats showed normal metaphase (Fig. 10). The results of chromosomal aberration in male rats' bone marrow cells treated with 4 mg/kg.bw after 8 weeks showed chromosomal chromatid deletions, dicentric chromosomes, fragment centromeric separation, and ring chromosomes (Figs. 11,12, 13).

Discussion

The changes in body weight in acrylamide treatments indicate that acrylamide affects body weight in addition to the histological changes that is in agreement with the findings of Wang et al. (2010).

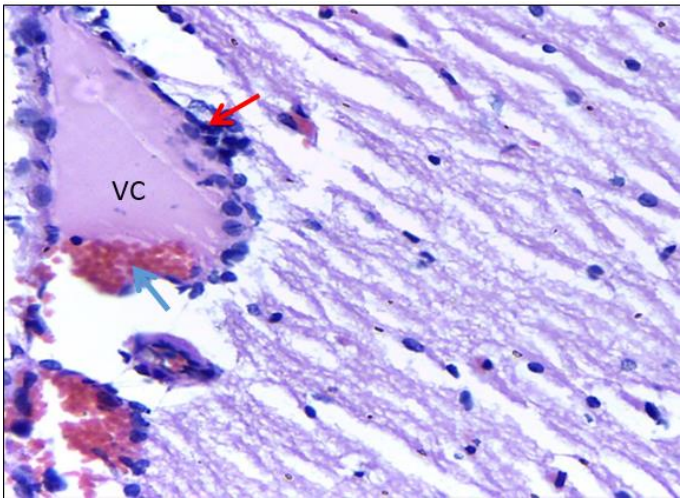

Fig.5. Cross section of Posterior gland of rats after 8 weeks post treatment shows vesicles with colloid (VC), hemorrhage (blue dart), inflammation of inflammatory cells (red dart) and decrease pytucytes, H&E, 400X.

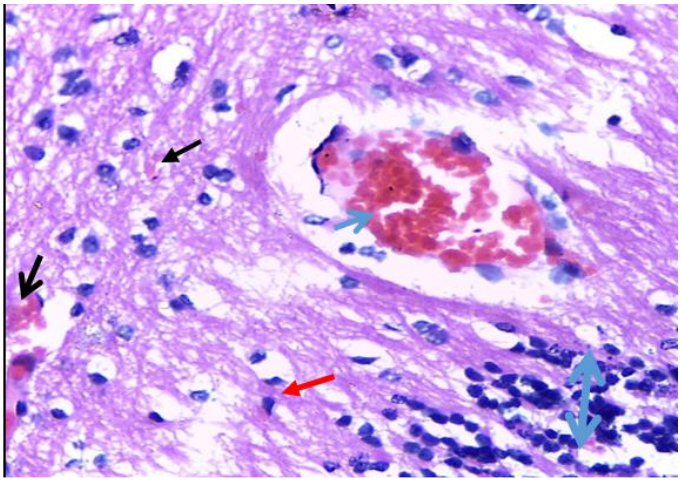

Fig.6. Cross section of Posterior and anterior Pituitary gland of rats after 8 weeks post treatment shows hemorrhage (black dart (bold), sever congestion (blue dart (small)), inflammation of in flammatory cells (blue dart), and pitucytes (red dart) embedded within connective tissue and normal capillaries (black dart), H&E, 400X.

Body weight is a non-specific indicator of chemical toxicity that may be used to investigate the impact of toxicity on rats' development (Somasundaram et al. 2017). The results also showed a decrease in the weight of the pituitary gland treated with acrylamide, which is consistent with the findings of Capen (1983), which could be attributed to the acceleration release of TSH granules that occurred by the thyroid. As a result of the toxicity, the pituitary glands' first reaction is the rapid release of performed path hormone granules from one gathering of the endocrine cells in the pars distalia.

The study's findings revealed an increase in T4 serum hormone and a decrease in TSH serum hormone, which are similar to the findings of Khan et al. (1999), who found a moderate dose-dependent rise in plasma T4 level and a small drop in TSH serum concentration after 7 days of acrylamide exposure. Our results were contrary to the results of Sharma (2008). As the thyroid hormones fair dominate the growth and development of tissues, cell respiration, total energy expense for a long way with basic rotation in all substrates, vitamins and hormones, including thyroid hormones itself so the

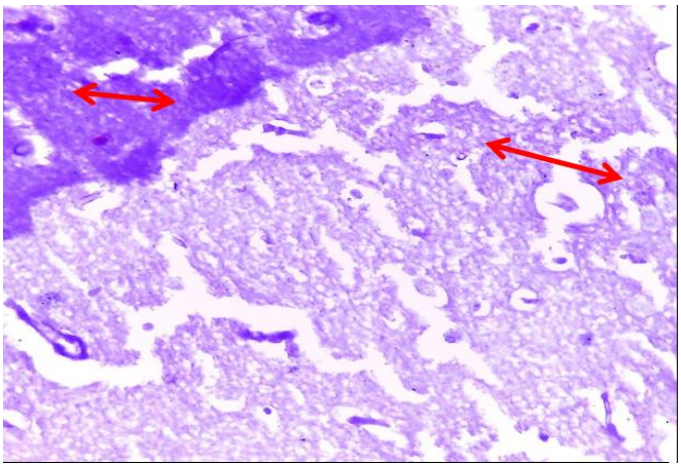

Fig.7. Cross section of Posterior and anterior Pituitary gland of control rats shows homogenous distribution of mucopoly saccharide an all cells within anterior and posterior regions (red dart), PAS, 400X.

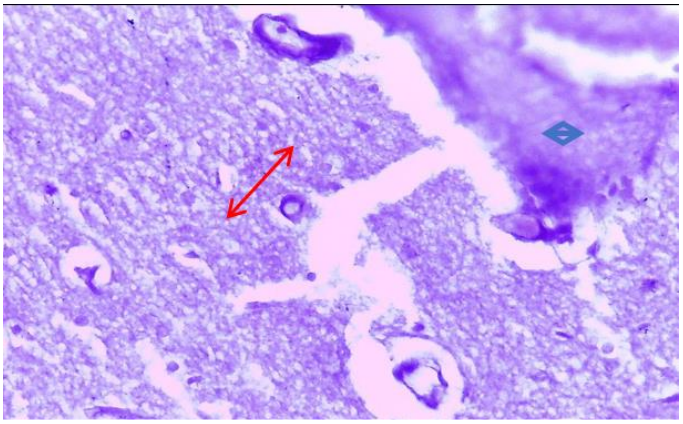

Fig.8. Cross section of Posterior and anterior Pituitary gland of rats after 8 weeks post treatment shows homogenous distribution of mucopoly saccharide in posterior region (blue), and moderate in anterior region (red dart), PAS, 400X.

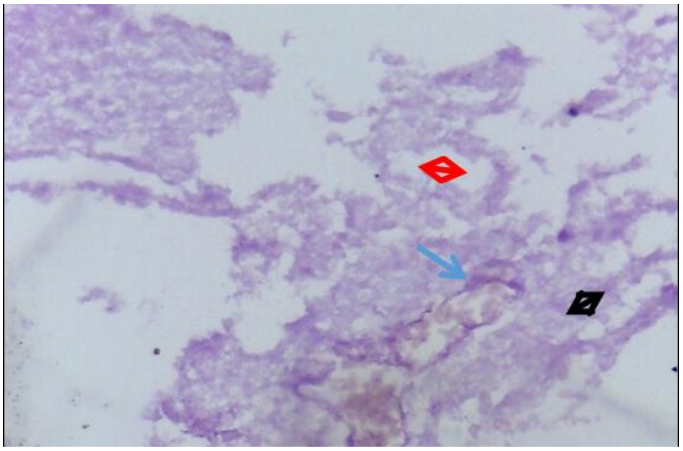

Fig.9. Cross section of Posterior Pituitary gland of rats after 8 weeks post treatment shows weak reaction of mucopoly saccharide (black), lose connective tissue (red), and congested blood vessels (blue) PAS, 400X.

differences syndrome as reductions of body weight, sluggish appearance, bizarre behavior, bulging of eyes along with decrease consuming of food and water.

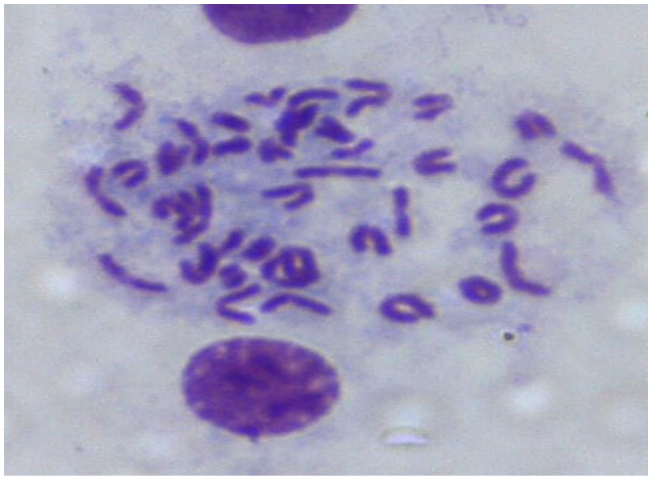

Fig.10. Metaphase figure of chromosomal aberration of control rats of bone marrow cells showing normal metaphase (Geimsa, 1000X).

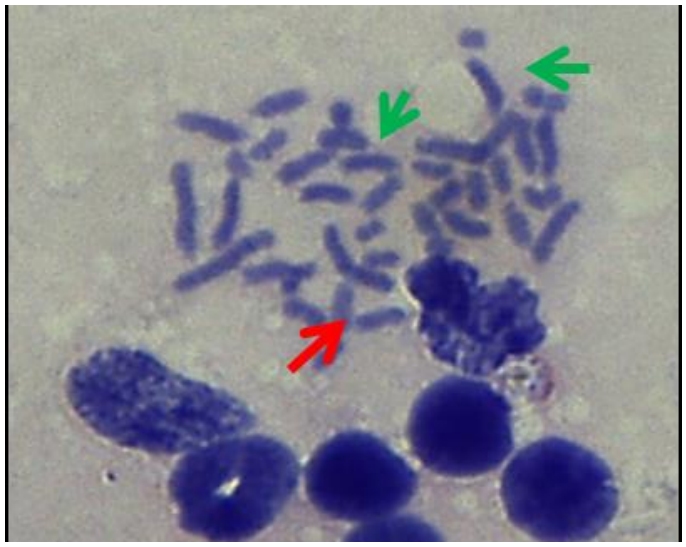

Fig.11. Metaphase figure of chromosomal aberration from treated male rats with 4 mg/kg.bw. after 8 weeks, showing ring chromosome (green dart), and centric separation (red dart). Geimsa,1000X.

Mannaa et al. (2006) found that treating female rats with ACR (50mg/kg.bw.) for 11 days resulted in a significant decrease in serum T3 and T4, which was consistent with our findings. Hogervorst et al. (2021) showed a relationship between acrylamide and glycidamide and cord plasma (TSH and free T4). The rate of free T4 to free T3 and insulin are important mechanisms underlying the relation between gestational acrylamide exposure and fetal growth because those markers were positively related to fetal growth. Lin et al. (2015) revealed a negative and frontier significant link between the acrylamide metabolite in urine and TSH. The acrylamide effects on the pituitary gland are poorly investigated. Many studies described that pituitary secretions efficiency has been revealed influenced by metals and this endocrine gland is an essentially sensitive target to cadmium toxicity (Cano et al. 2007). Cd modifies the autotrophs activity of the pituitary gland through genomic and morphological changes, leading to pituitary dysfunction. Whereas, some researches indicate that Cd can compete with calcium at the pituitary gland cell membrane and then changes the cytoarchitectural solidity of the gland (AL-okaily 2017). The histological examination of the pituitary gland of rats pointed to

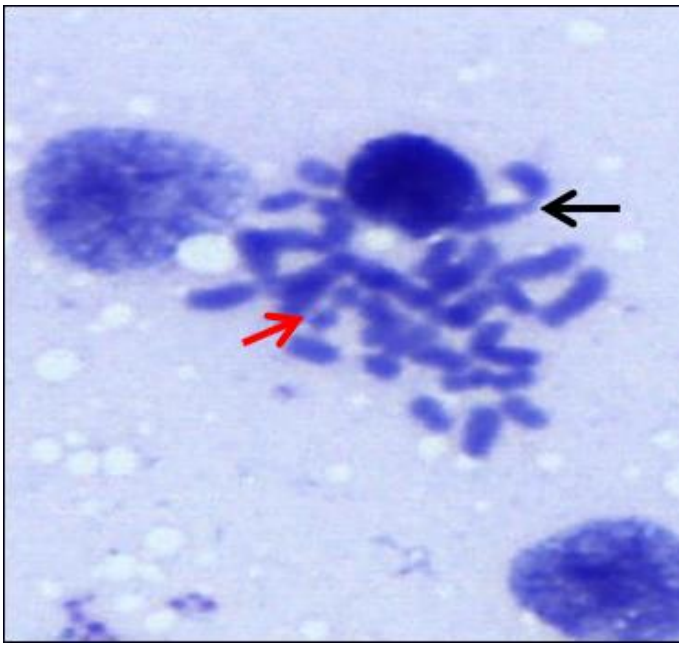

Fig.12. Metaphase figure of chromosomal aberration from treated male rats with 4 mg/kg.bw. after 8 weeks, showing ring chromosome (red dart), and dicentric chromosome (black dart). Geimsa,1000X.

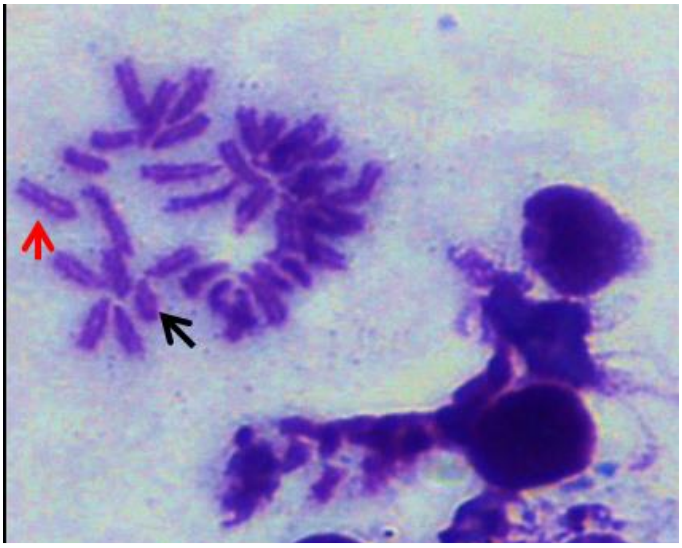

Fig.13. Metaphase figure of chromosomal aberration from treated male rats with 4 mg/kg.bw. after 8 weeks, showing deletion (black dart), and dicentric chromosome (red dart). Geimsa, 1000X.

modifications in the pars distalis together with few vacuolations, and an improvement of the cell membranes destructed by CdCl2 (Agarwal et al. 2014) and inhibits irregular cell growth and apoptosis via modulation of proteins regulating apoptosis (Bajilan & AL-naqeeb 2011).

Chromosomal aberration: At a high concentration of acrylamide, we detected chromosomal aberrations (Higashikuni et al. 1994). ACR is converted by cytochrome P450 to the epoxide glycidamide, which is subsequently the final DNA reactive clastogen in mouse spermatids (Adler et al. 2000). As a result, a chromosomal aberration caused by acrylamide might be caused by glycidamide binding directly to DNA and forming DNA adducts. Furthermore, Tyl & Friedman (2003) discovered that acrylamide and/or

glycidamide binding to spermatid protamines promotes gonadal cell death and sperm morphological defect.

In conclusion, ACR causes pituitary degeneration and histological changes and decreases the content of its polysaccharides, as well as the hormonal alteration in T4, and TSH concentration occurs. The cytological study also showed chromosome changes, such as ring chromosomes and dicentric chromosomes in all bone marrow smears in the treated rats.

References

Agarwal, A.; Virk, G.; Ong, C. & Du Plessis, S.S. 2014. Effect of oxidative stress on male reproduction. The World Journal of Men's Health 32(1): 1-17.

Adler, I.D.; Baumgartner, A.; Gonda, H.; Friedman, M.A. & Skerhut, M. 2000. Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatids of male mice. Mutagenesis 15(2): 133-136.

Al-Okaily, B.N. 2017. Histological changes in pituitary-testes axis in rats exposed to cadmium chloride: Protective role of Eruca sativa seeds. The Iraqi Journal of Veterinary Medicine 41(1): 76-85.

Bajilan, S.I. & Al-naqeeb, A.A. 2011. Effect of the aqueous extract of rocket (*Eruca sativa* L.) leaves on the histological structure of some organs in male mice. Journal of the College of Basic Education 17(70): 13-21.

Baum, M.; Loeppky, R.N.; Thielen, S. & Eisenbrand, G. 2008. Genotoxicity of glycidamide in comparison to 3-N-nitroso-oxazolidin-2-one. Journal of Agricultural and Food Chemistry 56(15): 5989-5993.

Borgeest, C.; Greenfeld, C.; Tomic, D. & Flaws, J.A. 2002. The effects of endocrine disrupting chemicals on the ovary. Frontiers in Bioscience 7(1-3): d1941-d1948.

Brisson, B.; Ayotte, P.; Normandin, L.; Gaudreau, É.; Bienvenu, J.F.; Fennell, T.R. & Bouchard, M. 2014. Relation between dietary acrylamide exposure and biomarkers of internal dose in Canadian teenagers. Journal of Exposure Science & Environmental Epidemiology 24(2): 215-221. Cano, P.; Poliandri, A. H.; Jiménez, V.; Cardinali, D.P. & Esquifino, A.I. 2007. Cadmium-induced changes in Per 1 and Per 2 gene expression in rat hypothalamus and anterior pituitary: effect of melatonin. Toxicology Letters 172(3): 131-136.

Capen, C.C. 1983. Functional and pathologic interrelationships of the pituitary gland and the hypothalamus. In *Endocrine System* (pp. 101-120). Springer, Berlin, Heidelberg.

Dearfield, K.L.; Douglas, G.R.; Ehling, U.H.; Moore, M.M.; Sega, G.A. & Brusick, D.J. 1995. Acrylamide: a review of its genotoxicity and an assessment of heritable genetic risk. Mutation Research/Fundamental and Molecular Mechanisms of Mutagenesis 330(1-2): 71-99.

Yeşim, Y.; Fatma Hümeyra, Y. & Aysun, T. 2016. Evaluation of Some Renal Function Parameters in Rats Treated with Acrylamide. ARC Journal of Animal and Veterinary Sciences (AJAVS). 2(1): 1-8.

Hammad, A.Y.; Osman, M.E.; & Abdelgadir, W.S. 2013. Effects of acrylamide toxicity on growth performance and serobiochemisty of Wistar rats. British Journal of Pharmacology and Toxicology 4(4): 163-168.

Higashikuni, N.; Hara, M.; Nakagawa, S. & Sutou, S. 1994. 2-(2-Furyl)-3-(5-nitro-2-furyl) acrylamide (AF-2) is a weak in vivo clastogen as revealed by the micronucleus assay. Mutation Research/Genetic Toxicology 320(1-2): 149-156.

Hogervorst, J.G.; Saenen, N.D. & Nawrot, T.S. 2021. Gestational acrylamide exposure and biomarkers of fetal growth: Probing the mechanism underlying the association between acrylamide and reduced fetal growth. Environment International 155: 106668.

Khalil, W.K.B.; Ahmed, H.H.; Hanan, F.; Aly, H. & Eshak, M. 2014. Toxicological effects of acrylamide on testicular function and immune genes expression profile in rats. International Journal of Pharmaceutical Sciences Review and Research 24(1): 143-151.

Khan, M.A.; Davis, C.A.; Foley, G.L.; Friedman, M.A. & Hansen, L.G. 1999. Changes in thyroid gland morphology after acute acrylamide exposure. Toxicological Sciences: An Official Journal of the Society of Toxicology 47(2): 151-157.

Konings, E.J.; Baars, A.J.; van Klaveren, J.D.; Spanjer, M.C.; Rensen, P.M.; Hiemstra, M. & Peters, P. W. J. (2003). Acrylamide exposure from foods of the Dutch population and an assessment of the consequent

risks. Food and Chemical Toxicology 41(11): 1569-1579.

Lyon, F. 1994. IARC monographs on the evaluation of carcinogenic risks to humans. Some Industrial Chemicals 60: 389-433.

Mahmood, H. 2014. Anatomical and histological study of pituitary gland of the rats in Iraq. Journal of Kerbala University 10(1): 221-228.

Mannaa, F.; Abdel-Wahhab, M.A.; Ahmed, H.H. & Park, M.H. 2006. Protective role of Panax ginseng extract standardized with ginsenoside Rg3 against acrylamide-induced neurotoxicity in rats. Journal of Applied Toxicology: An International Journal 26(3): 198-206.

Matoso, V.; Bargi-Souza, P.; Ivanski, F.; Romano, M.A. & Romano, R.M. 2019. Acrylamide: A review about its toxic effects in the light of Developmental Origin of Health and Disease (DOHaD) concept. Food Chemistry 283: 422-430.

Maan, A.A.; Anjum, M.A.; Khan, M.K.I.; Nazir, A.; Saeed, F.; Afzaal, M. & Aadil, R.M. 2020. Acrylamide formation and different mitigation strategies during food processing–a review. Food Reviews Internationa*l*, 1-18.

Nordin-Andersson, M.; Walum, E.; Kjellstrand, P. & Forsby, A. 2003. Acrylamide-induced effects on general and neurospecific cellular functions during exposure and recovery. Cell biology and Toxicology 19(1): 43-51

Pedreschi, F.; Mariotti, M.S. & Granby, K. 2014. Current issues in dietary acrylamide: formation, mitigation and risk assessment. Journal of the Science of Food and Agriculture 94(1): 9-20.

Somasundaram, D.B.; Manokaran, K.; Selvanesan, B.C. & Bhaskaran, R.S. 2017. Impact of di-(2-ethylhexyl) phthalate on the uterus of adult Wistar rats. Human & Experimental Toxicology 36(6): 565-572.

Tyl, R.W. & Friedman, M.A. 2003. Effects of cacrylamide on rodent reproductive performance. Reproductive Toxicology 17(1): 1-13.

Wang, H.; Huang, P.; Lie, T.; Li, J.; Hutz, R.J.; Li, K. & Shi, F. 2010. Reproductive toxicity of acrylamide-treated male rats. Reproductive Toxicology 29(2): 225-230.