Histopathological study on acrylamide induced testicular changes in adult male rats

Aisha D. Alalwani
Biology Department- Science Faculty for Girls - King Abdulaziz University- Jeddah- Saudi Arabia

ABSTRACT

Acrylamide (ACR) is a common chemical which is used in both industrial and laboratory processes. It is formed in heated starchy foods especially potato products. The present experiment was conducted to investigate the testicular toxicity of acrylamide in adult male rats. Twenty mature male wistar rats were used in this study. Rats were classified randomly into two groups; the first group G1 was received daily saline solution and kept as control. The second group G2 acrylamide was administration daily to animals at dose of 25 mg/kg for 6 consecutive weeks. The results indicated decreased significantly (P< 0.05) in relative weights of testes and body weights of rats treated with acrylamide. Histopathological lesions were also present in the testes of treated rats as vacuolar degeneration and disorder of germ cells, deformed spermatids and seminiferous tubules atrophy, necrotic sertoli cells and lack of spermatozoa in tubular lumen of epididymidis were observed in treated group animals. This may suggest partial depletion of germ cells and different physiology roles for germ cells subunits in spermatogenesis. These changes might affect to reproductive and sexual behavior.

Keywords: acrylamide, rats, testis, histopathology

I. INTRODUCTION

ACR is a widely used industrial chemical. It is a white, odorless, crystalline solid at room temperature with a molecular formula of C_3H_5NO **(Grivas *et al.*, 2002)**. ACR has been reported to be present in plant material such as potatoes, carrots, radish, lettuce, Chinese cabbage, parsley, onions, spinach and rice paddy (**Arikawa and Shiga, 1980)**, in sugar **(Sehultzova and Tekel, 1996)** and olives **(Friedman, 2003)**. ACR is found in carbohydrate-rich food prepared at high temperatures such as french fries and potato chips that are consumed by humans, consumption of these foods may result in significant human exposure to acrylamide where the formation of ACR is associated with high temperature (higher than 200^0C) cooking process of certain carbohydrate rich foods(**Rydberg *et al.*, 2003**).

ACR can undergo oxidative biotransformation by cytochrome P**450** (**Sumner *et al.*,1999**). The resulting metabolite is an epoxide derivative, that is glycidamide, which is more reactive towards DNA and proteins than the parent compound ACR (**Dearfield *et al.*, 1995**). The biological consequences of ACR exposure have chiefly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound (**McCollister *et al.*, 1964; Klaunig and Kamendulis , 2005 and Nuno *et al.*, 2008)** ,and produces peripheral neuropathy in animals **(Hashimoto and Aldridge, 1970).**

Subsequently, experimental exposure of rodents to ACR has also revealed a carcinogenic mode of action for this chemical **(Friedman, 2003)**. Orally consumed ACR is absorbed into the circulation then distributed to various organs, and reacts with DNA and essential enzymes **Baum *et al.*,(2008**) and **Rayburn and Friedman , (2010)** added that ACR causing several toxic effects as animal carcinogen and germ cell mutagen **Ghanayem *et al.*, (2005)**.

ACR is not genotoxic by itself but becomes activated to its primary epoxoide genotoxic metabolite glycidamide (GA) via epoxidation **Baum *et al.*,(2008),** *by* CYP2E1 which leads to the formation of GA-DNA and hemoglobin adducts **(Ghanayem *et al.*, 2005),** reproductive toxicant and carcinogen in animals **(El-Assouli, 2009).** However, **Butterworth, *et al.* ,(1992)** reported that ACR induce dominant lethal mutations in male rat germ cells and tumors in a variety of organs, including the scrotum, thyroid and mammary glands.

ACR-induced hepatotoxicity as the metabolism of ACR mediated through glutathione conjugation in the liver tissue **(Miller *et al*, 2004)**. Histopathological investigation revealed necrotic and degenerative changes in the liver of acrylamide treated rats **(EL-Bohe *et al.*, 2011)**. The present study was performed to determine the histopathological lesions effects of acrylamide on testis in male rats.

II. MATERIALS AND METHODS

a) Chemical

Acrylamide (ACR) purity (99.9%) was supplied from sigma chemical company. The applied doses were selected according to **Tyl and Friedman (2003).**

b) Experimental animals

The study was conducted accordance on the National Institutes of Health guidelines for the use of experimental animals. Wistar rats, a total of 20 males, weighting 220.00±7.88g. Obtained from the Animal House of the King Fahd Center for Medical Research, King Abdul Aziz University in Jeddah of Saudi Arabia. Rats were maintained on a 12 h light/dark cycle at 21 ± 1°C and 50 ± 10% humidity, and left several days of adaptation.

c) Animals Treatment

The animals were divided randomly into 2 groups: **Group1:** contained 10 rats which received daily 0.2 ml single interaperitoneal injection of saline solution.
Group2: contained 10 rats which received daily a single dose of 25mg /kg i.p. of ACR dissolved in 0.2 ml of saline solution.

d) Body and Relative testis Weight

The behavioral and morphological changes that have taken place were recording, and record the body weight of the rats weekly during the experimental periods. Rats were dissected 24 hours after the end of experimental period 6 weeks and recorded the changes in body weight of each animal, and the testis of all animal were dissected out and weighed **(Bancroft and Gamble, 2002; Hummdi, 2012).**

e) Histopathological examination of testis

Testis tissues were fixed in buffered formalin solution after sacrifice rats and immersed in neutral formalin solution. The tissues were dehydrated in an ascending grade of alcohol (ethanol), cleared in xylene and embedded in paraffin wax. Serial sections of 2 μm and mounted on slides. These sections were stained with hematoxylin and eosin (H&E), and histopathological changes were examined under a light microscope **(Bancroft and Gamble, 2002).**

f) Statistical analyses

Results are presented as means (M) ± standard deviations (SE). Multiple comparisons were performed using student's t-test, of absolute body weights and testes weights by comparing the control and experimental groups). Data were considered statistically significant at $p < 0.05$. All Statistical procedures were computed using SPSS 18.0 software.

III) RESULTS

a) Behavioral observations

Administration of ACR to male rats resulting in marked alterations in behaviour, revealing nervous manifestations (abnormal neurobehavior) in the treated group(G2) as ataxia, increased landing of the limbs, general emaciation. The severity of the clinical signs was dose and time dependant as these manifestations appeared on the 12th days of ACR treated group.

b) Body and testis weights

The body weight gained after administration of ACR were measured and compared. In the group treated with ACR, loss of body and testis weight compared to control.

Table1:Body weight and testes relative weight of experimental rats after 6 weeks for treatment with acrylamide.

*** P < 0.05 ,** P < 0.001**

Animals groups		Body weight		Testes weight
		0 day	6 weeks	
Control (G1)	Mean ±S.D	220.0000±7.88106	325.0000±6.82330	5.01±22
	P	-	P=0.777	-
Treated (G2)	Mean ±S.D	223.0000±7.71068	253.3000±4.88888	4.02±23
	P	-	P=0.003**	0.014*

c) Histological results

All rats in the control group showed normal histological pattern **(Figs.1a,b,c)**,whereas sections of testes of rats given ACR depicted severely damaged of somniferous tubules **(Figs.1d-2f)**. The following microscopical changes were considered as characteristic signs for the severely damaged tubules. The majority of seminiferous tubules exhibited, malformed, accompanied by hyaline material in tubular lumen and damaged interstitial tissue **(Figs.1d,e,f).** At the same time exfoliated spermatids appeared in the lumena due to cellular necrosis in some tubules **(Figs.1e,2b)** and congested dilated blood vessels **(Fig.2a)**. Some seminiferous tubules manifested grade damage that included disorganization of spermatogenesis cells and the damaged germ cells lifting off the basal lamina **(Fig.2c)**, separated and irregular surface tubules, associated with atrophoid tubules also manifested **(Fig.2b)**. Also leydig cells damage around seminiferous tubules were noted **(Figs.1f,2e).** Disorders of germ cells and detachment of spermatogonial cells started at periphery of seminiferous tubules of testes showed vacuolar degeneration changes in germinal epithelium **(Fig.2d).** In addition, many spermatocytes appeared with pyknotic nuclei ,which acquired deeply basophilic stain ability **(Fig.2a-e)**. Also destruction of most spermatogenesis' layers with absence of spermatozoa was clearly recognized in other seminiferous tubule, degenerative alteration in seminiferous tubules, maturation arrest in early and late stages of spermatids and numerous vacuoles of variable sizes in both the seminiferous tubules and interstitial connective tissues

(Figs.1f, 2d-f), as a demonstrated damaged of sertoli cells**(Fig.2e)**, deformed late spermatid and sertoli cell nuclei were observed when rats were ACR treated, loss of spermatozoa and late spermatid in tubular lumen seen in the seminiferous tubules and epididymis**(Figs.2b,d,e,f).**

IV) DISCUSSION

Abnormal neurobehavioral changes recorded in the present work in animals ACR treated. **Alao *et al*., (2010)** match with observed as affect locomotion, sexual behavior in male and female rats. Since, administration of ACR to male rats resulting in marked behavioral and morphological manifestations in present study may attributed to ACR neurotoxicity that causing hind limb dysfunction which lead to in ability to get food. In addition, ACR may cause alterations in thirst and hunger regulation centers in hypothalamus (**WHO, 1985**). **EL-Bohe *et al*., (2011)** added ataxia, increased landing of the limbs, general emaciation in rats given ACR. **Shukla *et al*., (2002)** found that exposure of rats to ACR caused hind limb paralysis in 58% of the animals, they attribute these findings to ACR neurotoxicity. In the current study, the administered doses of ACR were high compared with that estimated in cooked food which is as high as 70 µg per day by **Tareke *et al.*, (2002**). In the present study, the decreased testes weight of rats were similar to those confirmed by **EL-Bohe *et al*., (2011)** in rats and in human **(Hogervors *et al*.,2007)**.Also the present work appeared several histopathological lesions in the seminiferous tubules. **Adler *et al*., (2000)** recored that ACR is metabolized by cytochrome P_{450} to the expoxide glycidamide, which is then the ultimate DNA-reactive clastogen in mouse spermatide. Therefore, chromosome aberration by acrylamide might result from direct binding of glycidamide to DNA by making DNA adducts. Also **Tyl and Friedman,(2003)** observed that acrylamide and/or glycidamide binding to spermmatid protamines causes dominant lethality of gonadal cells and morophological abnormalities of sperms. One of the histological lesions observed in the present study was the formation of many multinucleated giant cells in atrophied somniferous tubules. Which accordance with **Gassner and Adler,(1996**) studies that the giant cells result from the inability of primary 4N spermatocytes to undergo meiotic division to generate haploid sperm cells, which undergo additional DNA replication giving rise to multinucleated gaint cells. **Cheville (2009)** reported that the vacuolations and responsible for collecting the injurious elements and preventing them from interfering with the biological activities of cells. However, ACR disturbs the gene expression related to spermatogenesis, which might result in reduced sperm reserves in epididymidis. Therefore, ACR perturbs the gene levels related to cell proliferation and cell cycle, which might result in abnormal histopathological features in reproductive organs observed in this study. The dose of ACR in the current work significantly reduced the sperm concentration in epididymidis. **Tyl *et al*., (2000)** observed that rats exposure to 2.0 mg/kg/day of ACR in drinking water for 10 weeks showed no observable effect level of reproductive toxicity, and they observed the histopathological lesions under the extreme condition, which is a dose of acrylamide at 60 mg/kg/days. which suggests that the sperm concentrations in epididymidis decreased in an acrylamide dose-dependent manner. Atrophy of seminiferous tubules observed in ACR treated group was similar to the earlier findings by **yang *et al*., (2005); Burek *et al*., (1980**). In addition to, ACR-induced degeneration of seminiferous tubules as observed in present study was also reported earlier by **McColliste, (1964**). Since, ACR treatment lowered testosterone level leading to degeneration of seminiferous tubules with sloughing of somniferous epithelium and spermatogenic cells (**Yang *et al*., 2005**). **Wang *et al*., (2010**) results also indicate that the epididymal sperm reserves decreased, suggesting partial depletion of germ cells. This may suggest different physiological roles for germ cell subunits in spermiogenesis and steroidogensis. In agreement with recent work **Wang *et al*., (2007)** found ACR decreased the production of sperm and leydig cells in male rats**.** Since the increased free radicals generated by ACR exposure in testes might have been damage leydig cells and affects the endocrine function of the testis (**Song *et al*., 2008**).

CONCLUISON

ACR caused marked alterations in body weight and animals behavior . The toxic effect of its consumption have been shown from this study based on the histopathological changes in testis tissues. These changes might affect on reproductive and sexual behavior. So, ACR exposure either occupationally or dietary must be restricted. In addition to, raising awareness of people about its hazards.

REFERENCES

Adler,I.D., Baumgartner,A., Gonda,H., Frieman,M.A. and Skerhut,M. (2000): 1-Aminobenzotriazole inhibits acrylamide-induced dominant lethal effects in spermatide of male mice.Mutagenesis,15:133-136.

Alao, O. A., Ashaolu, , J. O., Ghazal, O. K. and Ukwenya, V. O. (2010): Histological and biochemical effects of monosodium glutamate on the frontal lobe of adult Wistar rats, International Journal of Biomedical and Health Sciences, 6(4): 197-203.

Arikawa A, Shiga M.(1980): Determination of trace acrylamide in the crops by gas chromatography. Bunseki Kagaku. 1980;29:33–39.

Bancroft, J. D. and Gamble, M. (2002): Theory and practice of histological techniques.5th.Ed. Edinburgh. Churchill Livingstone Pub, pp 172-5.

Baum M. Loeppky R. Thielen S. and Eisenbrand G. (2008): Genotoxicity of glycidamide in comparison to 3-N-nitroso-oxazolidin-2-one. J Agric Food Chem.; 56(15):5989-93.

Burek,J.D., Albee,R.R., Beyer, J.E., Carreon, R.M. and Morden,D.C.(1980): Subchronic toxicity of acrylamide administered to rats in the drinking water

followed by up to 144 days of recovey. J Environ Pathol Toxicol., 4:157-182.

Cheville, N.F. (2009): Ultrastructural pathology: The comparative cellular basis of disease. 2nd ed. Wiley-Blackwell. A john Wiley of Sons, Inc USA.

Dearfield KL, Douglas GR, Ehling UH, Moore MM, Sega GA, Brusick DJ.(1995): Acrylamide: A view of its genotoxicity and assessment of heritable genetic risk. Mutat Res.;330:71–99. McCollister DD, Oyen F, Rowe VK. Toxicology of acrylamide. Toxicol Appl Pharmacol. 1964;6:172–81.

El- Bohi,K.M, Moustafa, G.G, El sharkawi,N.I. and Sabik, L.M. (2011): Genotoxic Effects of Acrylamide in Adult Male Albino Rats Liver, Journal of American Science, 7(1).

El-Assouli S. M. (2009): Acrylamide in selected foods and genotoxicity of their extracts, J. Egypt Public Health Assoc., 84(3-4):371-92.

Friedman M.(2003): Chemistry, biochemistry, and safety of acrylamide: A review. J Agric Food Chem.;51:4504–26.

Gassner,P. and Dler,I.D.(1996): Induction of hypooloidy and cell cycle delay by acrylamide in somatic and germinal cells of male mice.Mutat Res.,367:195-202.

Ghanayem,B.I; McDaniel, LB; Churchwell,MI; Twaddle,N.C; Snyder,R; Fennell,TR and Doerge,DR(2005): Role of CYP2E1 in the Epoxidation of Acrylamide to Glycidamide and Formation of DNA and Hemoglobin Adducts. *Toxicol. Sci. ; 88 (2): 311-318.*

Grivas S, Jagerstad M, Lingnert H, Skog K, Tornqvist M, Aman P. (2002): Acrylamide in food mechanisms of formation and influencing factors during heating of foods. Sweden: Swedish National Food Administration

Hashimoto, K. and Aldridge, W.N. (1970): Biochemical studies on acrylamide, a neurotoxic agent, Journal Biochemical Pharmacology, 19(9): 2591-2604.

Hogervorst, J.G., Schouten, L.J., Konings, E.J., Goldbohm, R.A. and Van den Brandt, P.A.(2007): A Prospective Study of Dietary Acrylamide Intake and the Risk of Endometrial, Ovarian, and Breast Cancer, Cancer Epidemiol Biomarkers Prev, 16 (11): 2304-2313.

Hummdi, L. A. (2012): Histopathological and Ultrastructural Changes in Renal Corpuscle of Female Rats Topical Application by P-phenylene Diamine, International Journal of Zoological Research, 8(3): 106-120.

Klaunig, J. E. and . (2005): Kamendulis L.MMechanisms of acrylamide induced rodent carcinogenesis. Adv Exp Med Biol. ;561:49-62.

Mc Collister,D.D., Oyen,F. and Rowe,V.K.(1964): Toxicology of acrylamide. Toxicol APP Pharmacol.,6:172-181.

Miller, M.J., Carter, D.E. and Sipes, I.G.(2004): Pharmacokinetics of acrylamide in Fisher-334 rats, Toxicology Program, University of Arizona, Tucson, Arizona 85721 USA.

Nuno, G.O.; Célia M.; Marta P.; Gonçalo G. D.; Vanda M.; Maria M. M.; Frederick A. B.; Mona I. C.; Daniel R. D.; José R. and Jorge F. G. (2008): Cytogenetic damage induced by acrylamide and glycidamide in mammalian cells: correlation with specific glycidamide-DNA adducts. J Agric Food Chem.;56(15):6054-60.

Rayburn J.R. & Friedman M. (2010): l-Cysteine, N-Acetyl-l-cysteine, and Glutathione Protect Xenopus laevis Embryos against Acrylamide-Induced Malformations and Mortality in the Frog Embryo Teratogenesis Assay. J Agric Food Chem.; *58* (20): 11172–1117*58*.

Rydberg P, Eriksson S, Tareke E, Karlsson P, Ehrenberg L, Törnqvist M.(2003): Investigation of factors that influnce the acrylamide content of heated foodstuffs. J Agric Food Chem.;51:7012–8.

Schultzova J, Tekel J.(1996): Acrylamide monomer occurrence in sugar. Dtsch Lebensm Rundsch. 1996;92:281–2.

Shukla, P. K., Khanna, V. K., Ali, M. M., Maurya, R. R., Handa, S. S. and Srimal, R. C. (2002): Protective effect of *Acorus calamus* against acrylamide induced neurotoxicity. Phytotherapy Research, 16: 256–260.

Song, H.X.,Wang,R., Geng,Z.M., Cao,S.X. and Liu,T.Z.(2008): Subchronic exposure to acrylamide affects reproduction and testis endocrine function of rats, Zhonghua Nan Ke Xue.may; 14(5):406-410.

Sumner SC, Fennell TR, Moore TA, Chanas B, Gonzalez F, Ghanayem BI.(1999): Role of cytochrome P450 2E1 in the metabolism of acrylamide and acrylonitrile in mice. Chem Res Toxicol.;12:1110–6.

Tareke, E. ; Rydberg, P. ; Karlsson, P.; Eriksson, S. and Tornqvist, M. (2002): Analysis of acrylamide, acarcinogen formed in heated foodstuffs", J. Agric. Food Chem.; 50:4998-5006.

Tyl RW, Friedman, M.A. (2003): Effects of acrylamide on rodents reproductive performance, Reprod. Toxicol., 17(1): 1-13.

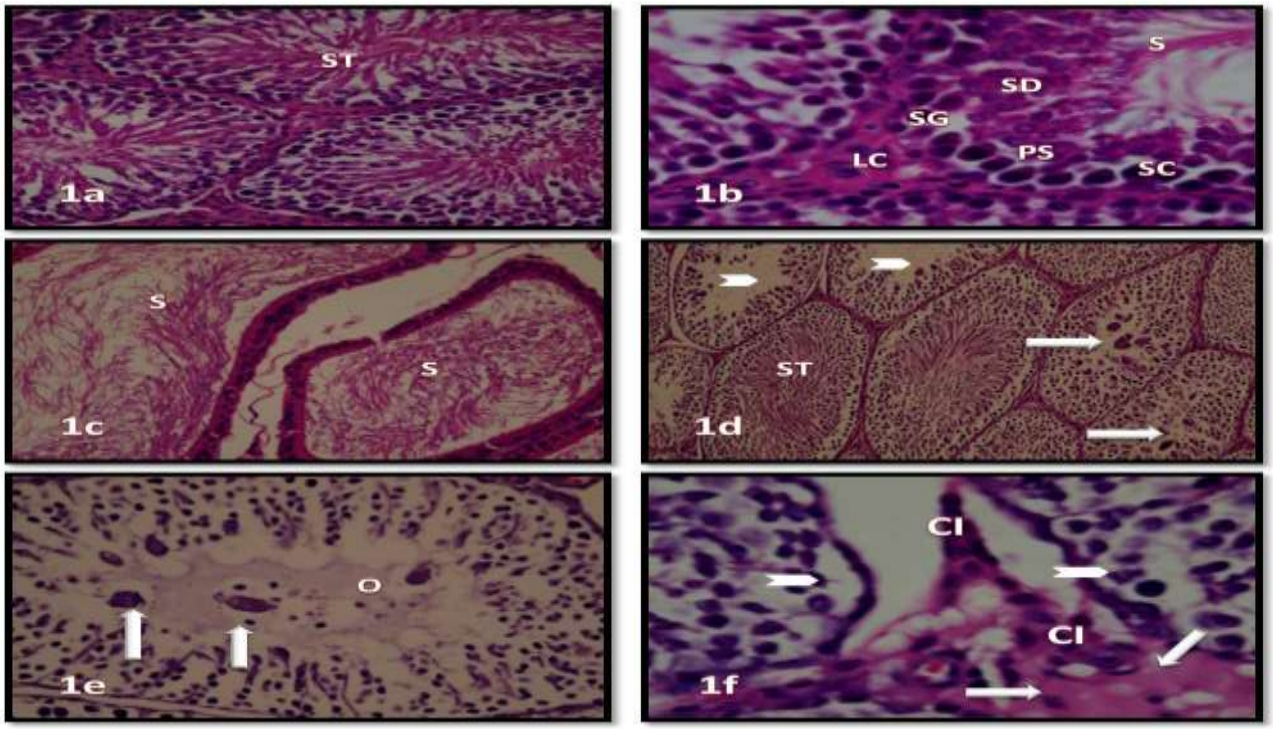
Tyl, R.W., Marr,M.C., Myers, C.B., Ross, W.P. and Friedman,M.A.(2000): Relationship between acrylamide reproductive and neurotoxicity in male rat. Reprod Toxicol.,14:147-157.

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

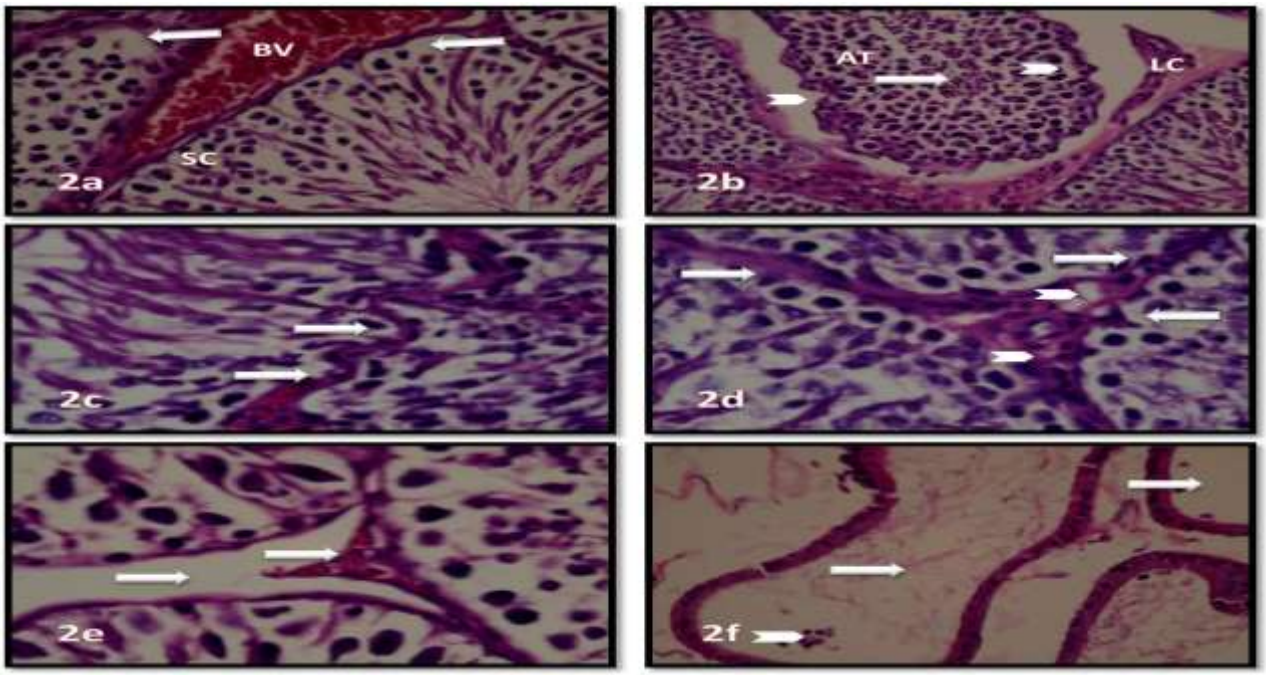
Wang,H.,Ge,J.Y.,Zhou, Z.C.,Wang,Z.C. and Shi, F.X.(2007): Oral acrylamide affects the development and reproductive performance of male rats, Zhonghua Nan Ke Xue.jun; 13(6):492-497.

WHO (World Health Organization) (1985): Acrylamide. Environmental Health Criteria 49. Geneva: WHO

Yang,H.J., Lee,S.H., Yong,J., Choi,J.H.,Han,C.H. and Lee, M.H.(2005): Genotoxicity and toxicological effects of acrylamide on reproductive system in male rats, J.Vet. Sci.,6(2):103-109.



Plate(1a-f):Cross sections of testicular of male Wister rats; H&E. (a-c) : testis sections of control rats (**G1**) :(**a**): showing the normal histological structure of the seminiferous tubules (**ST**) populated by spermatocytes and late spermatids surrounded the tubular lumen.;**x400**. (**b**):High power from previous section showing spermatogonium **(SG)**, primary spermatocytes **(PS),**spermatids **(SD),** tubular basal lamina with sertoli cell **(SC)**. Note myoid cell and narrow intertubular space contain interstitial leydg's cells (**LC**).;**x1000**. **(c):**showing epithelium cell of epididymidis and wide lumen full of spermatozoa(**S**). ;**x400. (d-f) sections of males treated testis with acrylamide (G2):(d):**showing enlarged seminiferous tubules (**ST**) populated by spermatocytes and spermatids surrounded. Note multinucleated giant cells and residual bodies in some tubular lumen(**arrows**), whereas appeared empty of some tubules (**headarrows**).;**x400. (e):**higher magnification of (fig.d): showing a disturbance in germinal epithelium of the seminiferous tubules , oedema (**O**), multinucleated giant cells and residual bodies in tubular lumen (**arrow**s). ;**x400.(f):**showing massive spermatogenic cell necrosis and detachment from the basement membrane (**headarrow**), and oedematous interstitum associated with cellular infilteration**(CI)** and eosinophilic droplates(**arrows**). ;**x1000**.



Plate(2a-f):Cross sections of testicular of male Wister rats treated with acrylamide; H&E.(G2):(**a**): showing dilation and congestion in blood vessels (**BV**), necrotic of germ cells in somniferous tubules (**arrows**). note sertoli cells (**SC**) remain intact. ;**x1000. (b):**showing atrophoid tubules (**AT**) filled with cellular debris and immature spermatids (**arrow**), irregular basement membrane (**headarrows**). Note damaged and necrotic leydg cellc (**LC**).; **x400.** (**c**): showing irregular and thickening of tubular basement membrane with massive spermatogenic cell necrosis(**arrows**) .;**x1000**. **(d):**showing massive germ cell and spermatogonial vaculations and degeneration, but still sertoli cell nuclei easily demarcated (**arrows**). Note vacuolated and degenerated leydg's cells(**headarrow**s).;**x1000**. **(e):**showing necrosis of germ cells and loss of tubular epithelium associated with outlines of deformed sertoli cell nuclei. Note congested and damaged interstitial tissue capillaries, leydgs cells necrotic (**arrows**). ;**x1000**. (**f**):showing epididymidis is empty from spermatozoa (**arrows**). Note residual bodies and some damage spermatocytes in tubular lumen(**headarrow**) .; **x400.**