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Original Article

Effects of acrylamide and children snack food on sex hormones nucleic acid and chromosomes of mature male Wister rats

Noorah Saleh Al-Sowayan

Department of Biology, Faculty of Science, Qassim University, P.O. Box: (30230), Buraydah (51744), Saudi Arabia

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ABSTRACT

The most dangerous problem a rise from plastic manufacture and the consumption of some types of foods lead to formation of acrylamide (ACR) potato chips during cooking, roasting or frying. Forty mature male Wister were divided into five equal groups, 1st group, control one, dosed orally dist. H_2O by stomach gavage, 2nd, group, dosed orally 12 mg/kg b.wt. acrylamide (low dose), 3rd group, dosed orally 24 mg/kg b.wt. (medium dose), 4th group dosed orally 36 mg/kg b.wt. (high dose) and 5th group fed on low packets of potato chips (65 gm each). All animals' groups dosed every other day for 65 days. The animals were used in chromosomal aberration were injected I/M colchicines, 90 minutes before sacrificed at the end of experiment each male rat epididymis was obtained and crushed for obtaining the seminal fluid for microscopic examination. Testes, seminal vesicle and prostate gland were weighted and fixed in neutral buffered formalin for histopathological examination ACR induced significant decrease in motility percentage sperm cell concentration. There was a significant increase in sperm abnormalities percentage. Moreover, the dosing ACR caused a significant decrease in the weight of testes, seminal vesicles and prostate gland of all treated groups in compared with control one. ACR administration causes a significant decrease in the levels of sera testosterone, FSH and cortisol while LH, estradiol and prolactin showed a significant increase in all treated groups. ACR effects revealed a clear cytotoxic activity where there were various chromosomal aberrations detected in bone marrow cells represented by centromeric attenuation, ring chromosome, end to end association and pulverized chromosomes. ACR caused a significant decrease in DNA & RNA content in testes in all treated groups. The histopathological changes of testes, showing atrophy in semine ferrous tubules and diffuse testicular degeneration with single layer of vacuolated spermatocytes and congestion of the interstitial blood vessels edema, few spermatocytes were seen in the lumen.

Key Words: Acrylamide, prostate glands, FSH, E2, cortisol, prolactin, DNA, RNA, chromosomal aberration.

INTRODUCTION

Acrylamide (ACR) monomer is found in certain foods cooked at high temperatures. ACR is thought to be found in food principally from the interaction of amino acid aspragine with glucose or other carbohydrates. ACR has been extensively investigated and has a large data base of very complex toxicity, toxicokinetic, toxicodynamic and physiological parameters alteration. ACR has been produced since the 1950 by hydration of acrylonitrile. It has used as flocculent for clarify-water and as flow control agents in oil well-preparations other major uses are in soil stabilization, in grout for repairing sewer and man hole and in acrylamide gel used in biotechnology laboratories. ACR is carcinogenic in standard bioassay in rats via drinking water producing increased incidence in a number of benign or malignant tumors in a variety of organs (thyroid, adrenal and testis) [1]. Reproduction is the physiological process that ensures the continuation of species. Where, the existing genetic material is passed to the next generation. Reproductive toxicity includes adverse effects on physiological sexual functions and fertility in adult male and female as well as developmental toxicity in off spring [2]. Recently exposure to ACR in food stuffs has become a worldwide concern because of its generation in a variety of fried and oven backed foods during cooking through maillard reactions. It was first detected in certain human feed by Swedish National Food Administration (CSNFA) [3]. The average human intake is estimated to be

**Corresponding Author E-mail: drnoorahknaajl@yahoo.com*

0.4 mg/kg/day or may vary widely from 0.3 to 2 mg/kg /day or may reach even 5 mg/kg /day at the 99% of human [4]. ACR is a chemical intermediate used for production of polyacrylamide. In the same study they have applied an analytical study showed the food items that are the major contributors in western diet to total exposure to ACR: 13-39% coffee, 10-30% bread and rolls (toast), 10-20% sweat biscuits [5]. ACR as a versatile chemical that formed in food from sugars and amino acids those are naturally present in food. It does not come from food packaging or environment [6]. ACR is used in several uses as cosmetic additives (e.g. creams, body lotions & shampoo) direct and indirect food additives such as in paper and paper board food packing and coating ACR is also a component of tobacco smoke, which indicates that it can be formed by heating of biological materials [7].

ACR has scientific application, as water purification, sewage treatment or processing and gel electrophoresis [8]. Reaction between carbohydrates and amino acids forming a brown pigment when the author heated a mixture of glucose with amino acids, lysine. The reaction has come to be known as either the maillard reaction or non enzymatic browning. Since then, many of wonderful odors that reaction we associate with coffee, black breads, butter, chocolate, grilled meat baked and French fried potatoes have been shown to be due to chemicals produced by the maillard reaction [9]. The formation of ACR in food is associated with high temperature (higher than 200 ^{o}C) cooking process in certain carbohydrate rich foods (backed or fried especially chips or fries. ACR formation is affected by several processing parameters such as heating temperature, pH, addition of components that bind water, cooking time and also water content [10]. ACR is formed in high temperature cooking such as frying, roasting, but boiling and steaming do not typically from ACR. ACR is found mainly in food made from plants such as potato products, grain products or coffee. ACR does not form in dairy, meat or fish products [6]. Glycidamide (GA) is formed via expoxidation by cytochrome P450 and its thought to be the active metabolite playing a central role in ACR genotoxicity because when the mutagenicity of ACR and GA have been compared and has been more potent [11]. The estimated plasma half life for ACR following oral 20 mg/kg) or I/P (50 mg/kg) administration in male spargue. Dawlly rats was approximately 2 hrs for both ACR and glycidamide following single or repeated exposure [12]. The ingested ACR is taken up into the circulation and excreted mainly with sixty percentage of a dose 0.44 mg contained in a meal of volunteers was recovered from urine within 72 hrs. [13].

The Aim of Work: This study testing the effect of acrylmide on the fertility of mature male Wister rats. Effect of acrylamide (ACR) on testes and accessory glands weights, epididymal semen analysis, serum level of sex hormones, The cytogenotoxic effects on bone marrow chromosomes, the quantitative levels of nucleic acid (DNA & RNA) of testes and Histopathological examination of testes, prostate gland and seminal vesicle.

MATERIAL AND METHODS

Experimental design: Forty mature male Wister albino rats (180-200 gm weight), were obtained from the laboratory animals unit, The Animal House in the College of Pharmacy King Saud University Saudi Arabia, were classified into five groups, eight in each. Accommodate in separated cage with feed & water ad libtum. The light cycle was 12/12 hrs dark / light daily. Experimental design was explained in table (1).

Acrylamide (Acrylic acid amide $-C_3H_5$ NO): Acrylamide was obtained from sigma laboratories, Cairo, Egypt. The median lethal dose (LD_{50}) of ACR in rat 120 mg/kg b.wt.**(14)**. The treated groups with ACR was dissolved in 2 ml dist. H_2O and dosed orally by stomach gavage every other day for 65 days.

Children snacks (Potato chips): Produced by chipsy for food industries Co.S.A.E., consists of fresh potatos, vegetable oil and flavor. The fried chips were packed hot in aluminum foil bags coated from inside with plastic coat. The chips group received two packets of potato chips each one weights 65 gm every other day for 65 days beside normal balanced ration Table (1).

Effect of ACR on mature male fertility: The effect of acrylamide (ACR) on male fertility was assessed using sexual organs, weight, epididymal spermatozoal examination according to technique adapted by Bearden and Flquary [15].

Physiochemical analysis of sex and fertility hormones in serum for evaluation of male fertility: Blood samples were collected during scarifying in tubes without anticoagulants for colleting the serum to detection testosterone, FSH, coat: sol, LH, estradiol and prolactin hormones **[16]**.

Chromosomal analysis: Chromosomal preparations were carried out according to Yosida and Amano [17].

Determination of RNA & DNA content in testicular tissues: Preparation of testicular tissue homogenate, four rats of each group. Testes were removed. Testes were homogenized in ice bidistilled water. The extraction of nucleic acid carried out according to Melmed et al. [18], RNA, [19] and DNA [20].

Histopathological examination: Specimens from the testes and prostate glands were collected after necropsy and fixed in 10% buffered neutral formalin [21].

Statistical analysis: The obtained data were analysed and graphically represented using the statistical package for social science (SPSS, 15.0 software) obtaining mean and standard error. The data were analysed one way ANOVA to determine the statistical significance of differences among groups [22].

RESULTS

Effect of acrylamide (ACR) on testes and accessory glands weights: There was significant decrease in the mean weights of testes of all treated groups comparing to control, table (2), Fig. (1). Seminal vesicles and prostate gland showed significant decrease (Table, 2) and Fig. (2).

Effect of ACR on epididymal semen analysis:
Sperm cell concentration and motility percent: Concerning to the effect of ACR on sperm motility and concentration (Table, 3) revealed significant decrease in both parameters in all treated groups.
Total sperm abnormalities percent: A significant increase in the percentage of sperm abnormalities in all treated groups comparing to control group (Table 3 and Fig. 3, 4, 5, 6 and 7).

Effect of ACR on serum level of sex hormones in mature male Wister rats: The oral administration of ACR to mature male rats effects on testosterone, FSH and cortisol were significantly reduced while significantly increase in estradiol (E_2), LH and prolactin (PRL) in compared with control group. The high dose of ACR effect on the mature male rats revealed that highly significant decrease in the levels of testosterone, FSH and cortisol while the estradiol, LH and PRL showed highly significantly increased compared with the control levels. The ships group clarified significant reduce of testosterone, FSH & cortisol while significant increase of E_2 , LH and PRL compared with control levels (Table 4).

The cytogenotoxic effects of ACR on mature male Wister rats on bone marrow chromosomes: The cytogenotoxic effects of ACR on the mature male wister rats dosed orally for 65 days every other day, showed several types of chromosomal aberrations either numerical as pulverization and haploidy and structural as centeromeric attenuation, sticky chromosomes, ring chromosomes, end to end association chromosome. In chips group found structural aberration only as ring chromosomes, end to end association and chromosomal breaks in compared with control group (Table 5), (Fig. 8, 9, 10, 11, 12 & 13).

The effect of acrylamid on the quantitative levels of nucleic acid (DNA & RNA) of testes:
Deoxy ribonucleic acid (DNA) in testes: The table (6) that revealed there was significant decrease in quantitative level of DNA in ACR treated and chips groups comparing with control group.

Ribonucleic acid (RNA) in testes: The quantitative levels of RNA in testes showed significant decrease in ACR treated groups. The significant decrease difference was clear between low dosed and chips group (Table 6).

Histopathological examination the effects of ACR on the mature male Wister rats:
Testes: The testes of animal of all groups were well observed immediately after scarifying. In all treated groups the testes showed atrophy ranged from moderate (low and medium dose) to sever (High dose and potato chips groups) compared to control (Fig. 14-17).

Seminal vesicle & prostate gland: The seminal vesicle of all male albino rats of treated group and control were well observed immediately after scarifying of rats. It showed sever shortening in both right and left sides of seminal vesicle in all treated groups comparing with control (Fig. 18 & 20). Moreover, the prostate gland of all treated groups and control showed changes in the normal gross morphology as sever atrophy in all parts of prostate (Lateral, dorsal and ventral (Fig. 21 & 23).

DISCUSSION

Since the 1970 acrylamide has of polyacrylamide and was a major cause of reported occupational poisoning [23]. ASR is a reactive highly water soluble monomer frequently used in polymer industry the cosmetics, paper and textile industries. Noso a day the fast food problems, become one of the most urgent topics in our fast daily life all over the world. Fast foods are widely used and increasing day after the other by all ranks of our society. It was reported that ACR is an industrial chemical with annual worldwide production estimated at more than 300 million kg. Concern

about human toxicity from ACR exposure [24]. In these study evaluated the effect of ACR at doses of (12, 24 & 36) mg/kg b.wt. by oral gavage every other day besides another grop administered two packets of potato chips (65 gm each) per day, wister rats fertility. Regarding the effects of ACR on weights of testes and accessory glands (seminal vesicle & prostate glands) revealed that there was a significant decrease in their weights table (2). This result was agreement with those recorded by Hye et al. [25] who found that oral administration of ACR at doses of 45 and 60 mg/kg for 5 days resulted in a significant decrease in testes weight and this condition may be attributed to the reduction of hormonal level of testosterone. Regarding, the result to of this study revealed that the epididymal sperm characters was significant decrease in progressive sperm motility and sperm count in ACR (12, 24 & 36 mg/kg b.wt.) with concurrent increase in the sperm abnormalities in the group treated with 36 mg/kg b.wt. for 65 days if compared with the control groups. In the previous treated groups we observed a found highly significant decrease in progressive sperm motility and sperm count and increase in sperm abnormalities table (3) and Fig. (3-7). These results agree with that obtained by these authors (26 & 27). The ACR was reduced the half number of sperm in the couda epididymis due to ACR treatment causes cell cycle delay in spermatogenesis [25]. Contrary to this study that observed no significant effects on the epididymal sperm motility or concentration in the rat testes.

These variations from our results may be attributed to the difference of species. Another possible explanation for our findings the reduction in the percent of motility and sperm cell concentration [27]. ACR acts through variety of biochemical mechanism that includes effects on the motor protein kinesin which would effect meiosis and mitosis, axonal transport and the sperm flagellar activity that greatly affect on the sperm motility. Alkylation of protein SH groups such as protamine in the sperm nucleus and protein SH groups in the sperm tail could also affect the sperm penetration causing pre-implantation losses –regarding the higher dose of ACR. These results were confirmed by histopathological finding. ACR significantly decrease the level of testosterone in serum this was in table (4). This hormone was the major sex hormone that is essential for male reproductive function [29]. It is synthesized from cholesterol in testis which controlled by FSH and LH through activation of adenylated cyclase enzyme, so the suppression testosterone production will enhance the increase in the level of LH hormone and that may be attributed to the feedback mechanism in which the lower testosterone hormonal level in serum stimulates the pituitary gland to secrete LH hormone. The effects of ACR resulted in more increase in LH level in rat sera [30].

A significant reduces of testosterone, FSH and cortisol and significant increase in LH, estradiol and prolactin were recorded in groups treated with ACR, but highly significant decreases of testosterone, FSH and cortisol and highly significantly increase in LH, estradiol and prolactin were observed in groups treated with ACR higher and medium doses. The increased serum L.H level is concordant with results [31]. Concerning of cytogenetoxic effects of ACR on bone marrow cells. This study showed that all treated groups had an obvious increase in both structural and numerical types of chromosomal aberrations harmony with the findings in FAO/WHO [32], which reported that ACR induces chromosomal aberrations sister chromatide exchange in mammalian cells in the absence of metabolic activation. The ACR induce chromosomal aberrations (breaks & gaps) and decrease mitotic index. Concerning with aberrations of bone marrow in thee studies showed significant increase in chromosomal aberration in bone marrow cells in groups treated in addition to chips group (Table, 5 and Fig. 8 – 13) in compared with control group. Chromosomal aberration which represented by centromeric attenuation and end to end association in agreed with the authors [33].

Who mentioned that the synthetic chemicals such as ACR resulted in the induction of numerical chromosomal aberrations (mainly aneuploidy) and the formation of DNA adducts [34]. Another speculation reported by author [24] mentioned that mechanisms of ACR must be involved in induction of the cytogenetic end points. It may be attributed to the increasing in the vulnerability of cells to oxidative damage, thus leading to DNA breaks. The effect of ACR on the quantitative levels of DNA & RNA of testes were recorded in table (6) revealed there was significant decrease in quantitative levels of DNA and RNA in all treated and chips groups but highly significant in higher dose level. The previously results of authors [35]. Confirmed our results which revealing that ACR caused reduction in the quantitative levels of DNA & RNA in testes of the four treated groups (Table 6).

Regarding histopathological findings of different male reproductive organs (tastes, seminal vesicles and prostate glands) treated with ACR revealed disturbed spermatogenesis, atrophy in the somniferous tubules with degenerated spermatogonia and spermatocyte, multinucleated giant cells with degenerated and increased

spermatocyte in compared with control (Fig. 14-23). These pathological findings are similar to that by author [13]. This testicular pathological alteration may be due to xenoestrogenic properties of ACR that can inhibit testicular growth.

CONCLUSION

This paper results clarify that ACR could be threat human health through the direct or indirect effects on animals an animal products and human through many mechanisms. The ACR which found in high concentration in potato chips and grain- based food (snacks foods) so dangerous. The recommended procedure to study and avoid the dangerous effects of these chemicals could be as follow: A great attention to organic chemicals and waster of all origins where the recycling of industrials effluents as well as disposal and treatment. Strictly control on children snacks foods used package plastic coated. Regular monitoring of the level of ACR in environment and baby foods (milk, condensed and concentrated milk). Coordination between all related ministries (agriculture, health, industry, education and military) to active environmental, animal and human and children health safety.

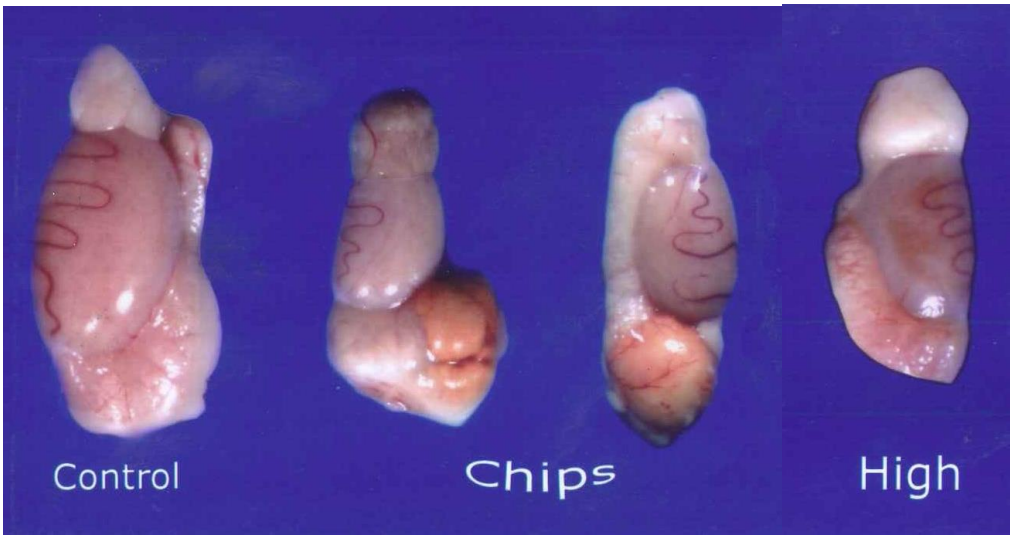

Fig. (1): Testis of mature male wister albino rats of ACR treated animals showed atrophy and little congestion but chips group showed unknown yellowish white structure.

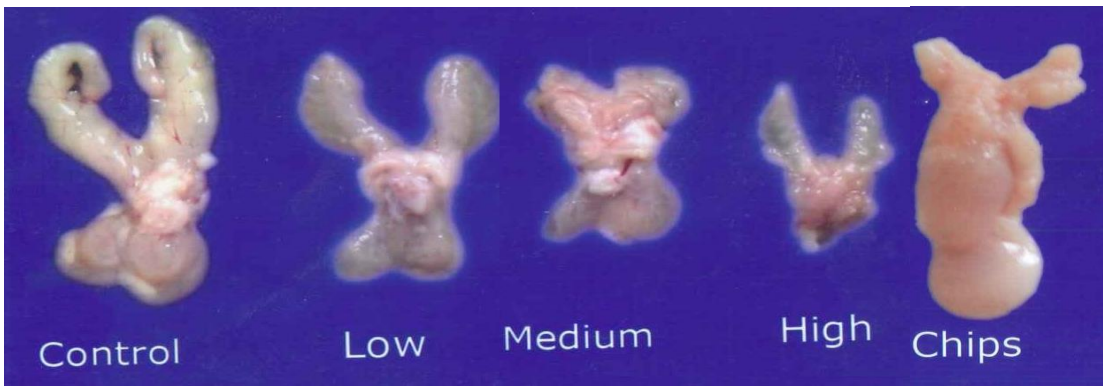

Fig. (2): Seminal vesicles and prostate gland showed different degrees of atrophy in ACR dose dependant manner of mature male wister albino rats.

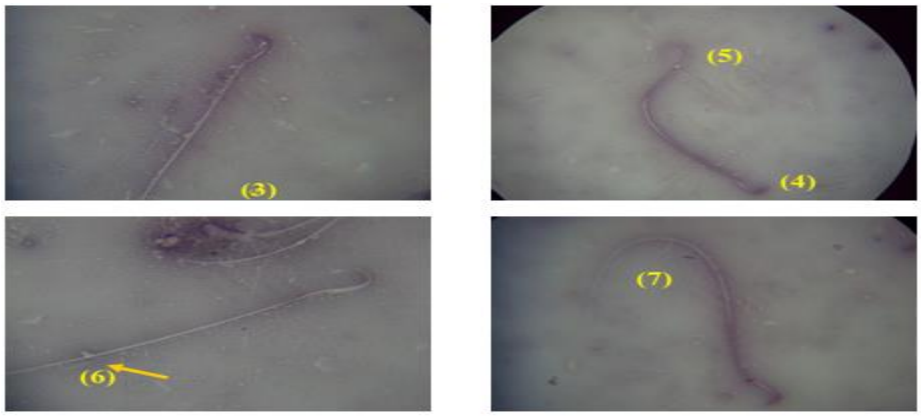

Fig. (3): Spermatozoa of normal mature male wister rats revealed normal head, middle piece and tail of control group.

Fig. (4): Spermatozoa of mature male wister rats treated with ACR at dose level 36 mg/kg b.wt. every other day for 65 days. The sperm abnormalities as A-lengthen and pointed hock shape.

Fig. (5): Spermatozoa of mature male wister rats treated with ACR at dose level 24 mg/kg b.wt. every other day for 65 days. The sperm abnormalities, A stunted sperm B.coiled tail.

Fig. (6): Spermatozoa of mature male wister rats treated with ACR at dose level 12 mg/kg b.wt. every other days for 65 days. The sperm abnormalities showed as protoplasmic droplets tail.

Fig. (7): Spermatozoa of mature male wister rats treated with ACR at dose level 36 mg/kg b.wt. every other days for 65 days. The sperm abnormalities showed as curved tail.

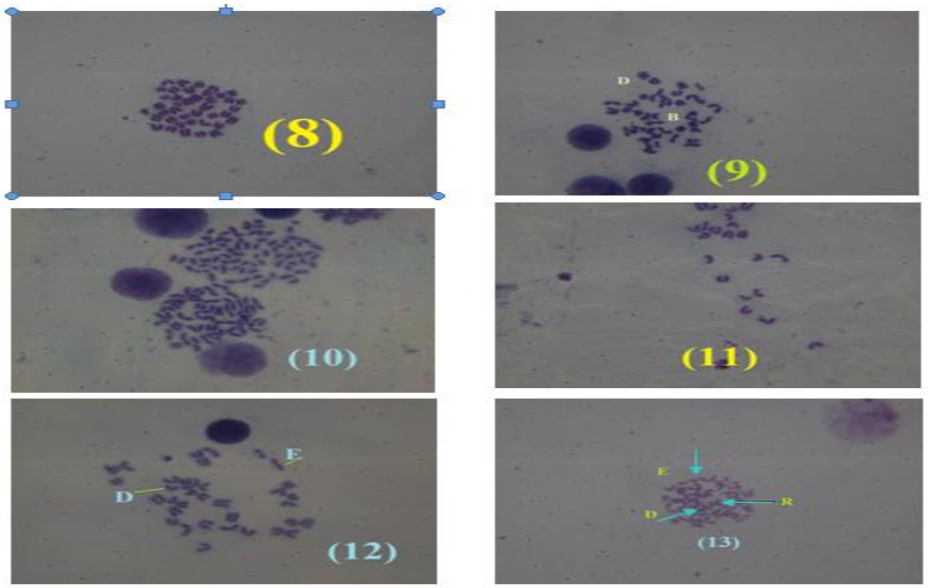

Fig (8): Normal chromosomes number and profile of metaphase spread from bone marrow of mature male wister rat (2n = 42 xy) (Giemsa stain) (Bar = 100 µm).

Fig. (9): Metaphase spread from bone marrow of mature male wister rats treated with low dose ACR for 65 days every other day, showing chromosome break (B) and chromatide deletion (D) (2n = 42 xy) (Giemsa stain) (Bar = 100 µm).

Fig. (10): Metaphase spread bone marrow of mature male wister rats treated with medium dose of ACR every other day for 65 days, showed pulverized chromosomes. (2n = 42 xy) (Giemsa stain) (Bar = 100 µm).

Fig. (11): Metaphase spread bone marrow of mature male wister rats treated with high dose of ACR showing haploid number of chromosomes. (2n = 42 xy) (Giemsa stain) (Bar = 100 µm).

Fig. (12): Metaphase spread bone marrow of mature male wister rats treated with chips group, showed, chromatid deletion (D), end to end association (E). (2n = 42 xy) (Giemsa stain) (Bar = 100 µm).

Fig. (13): Metaphase spread bone marrow of mature male wister rats treated with chips group, showed ring chromosomes (R) and end to end association and chromosomes breaks. (2n = 42 xy) (Giemsa stain) (Bar = 100 µm).

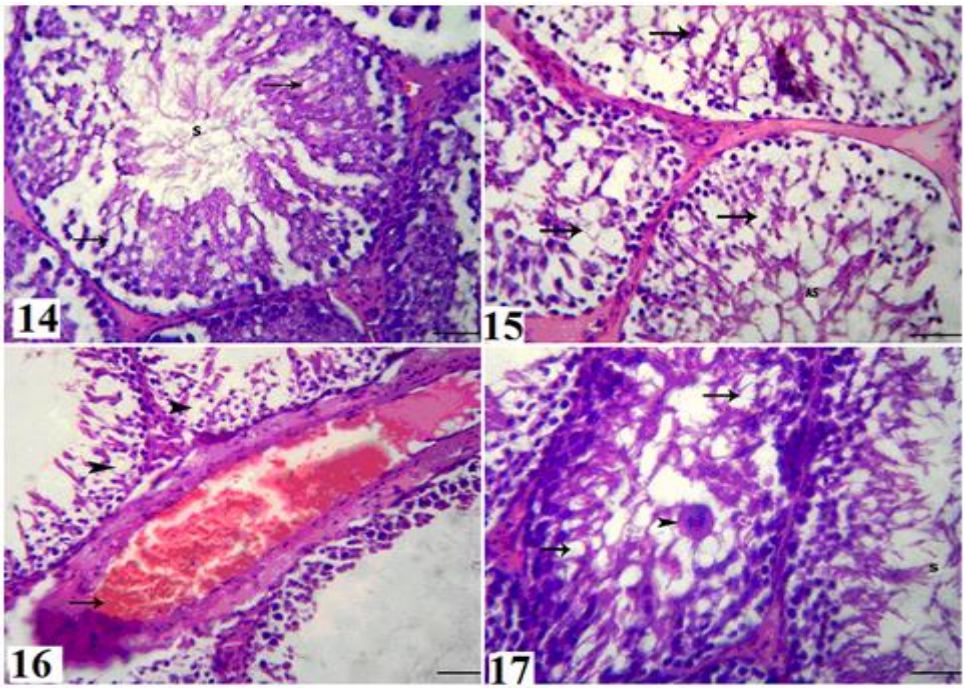

Fig (14-17): Testis from: 2nd group shows mild vacuolation in the spermatocytes (arrows) and normal spermatogenesis (S) (14). 4th group shows severe testicular degeneration with vacuolation of the lining epithelium (arrows) and incomplete spermatogenesis (AS) (15) and congestion (arrow) (16). 5th group shows mild testicular degeneration with vacuolation (arrows) and spermatid giant cells (arrowhead) and moderate spermatogenesis (S) (17). **HE x Scale bar = 50 µm.**

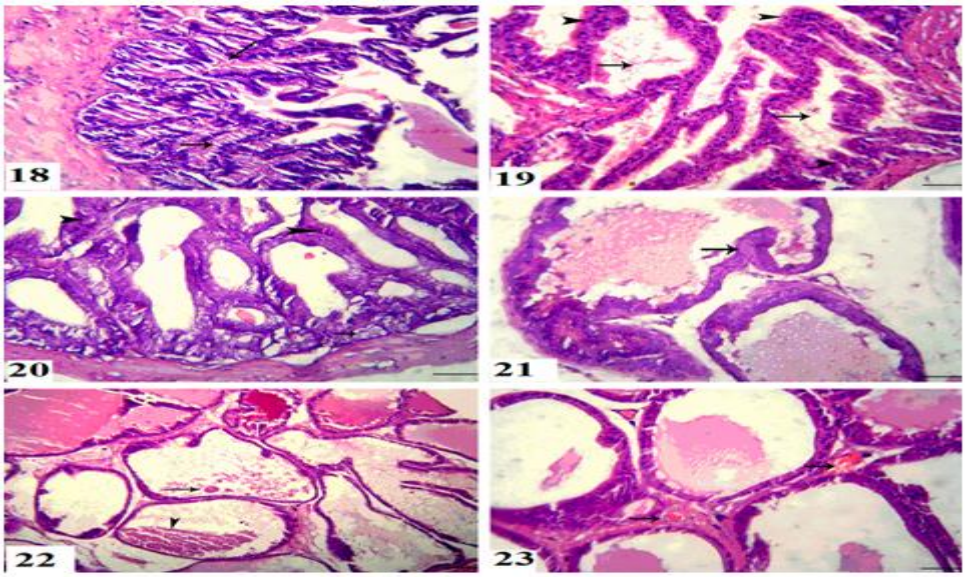

Fig (18-23): Sexual organs from: Seminal Vesicle (18-20): 2nd group shows mild vacuolation in the lining epithelium (arrows) (18). 3rd group shows necrosis (arrowheads) and inactive lining epithelium (arrows) (19). 4th group shows focal nuclear stratification (arrowheads) (20). Prostate gland (21-23): 2nd group shows normal acini with slight folding and hyperplasia in the lining epithelium (arrow) (21). 5th group shows eosinophilic granular material (arrowheads) and corpora amylacea (arrow) (22). 4th group shows congestion and edema in the interstitial tissue (arrows) (23). **HE x Scale bar = 50 µm.**

Table (1): Experimental animal designs N = 8.

Group / Treatment	Treatment	Dose & pattern of dosage
Control	2 ml of dist. H_2O, stomach	
Low dosed	ACR, orally by oral dosage, stomach gavage every other days	12 mg/kg b.wt. (1/10 LD_{50}) in 2 ml dist. H_2O for 65 days
Medium dosed	ACR, orally by oral dosage, stomach gavage every other days	24 mg/kg b.wt. (1/20 LD_{50}) in 2 ml dist. H_2O for 65 days
High dosed	ACR, orally by oral dosage, stomach gavage every other days	36 mg/kg b.wt. (1/20 LD_{50}) in 2 ml dist. H_2O for 65 days
Potato chips		2 packets of chips/ days (65 gm each) every other days for 65 days

Table (2): The effect of ACR on treated groups (low, medium, high) at doses of (12, 24 and 36) mg/kg b.wt./ day and chips group every other day for 65 days on the weights of testes seminal viseles and prostate gland of mature male wister albino rats.

Groups / Parameters	Testes Weight / gm	Seminal vesicle & prostate weight/ gm.
Control	4.85 ± 0.15 [a]	4.10 ± 0.05 [a]
ACR	2.40 ± 0.20[b]	1.90 ± 0.19b [c]
Medium dosed ACR	1.90 ± 0.09 [c]	1.55 ± 0.08 [bc]
High dosed ACR	1.25 ± 0.09 [c]	1.25 ± 0.20b
Chips	1.80 ± 0.30 [c]	1.50 ± 0.10[c]

Means within the same column in each item carrying different superscripts are significantly difference at P≤0.05

Table (3): The effect of ACR in treated groups (low, medium, high) at dose of (12, 24 and 36) mg/kg b.wt./ day and chips group every other day for 65 days on the motility %, sperm cell concentration and total sperm abnormalities percentage of mature male wister albino rats.

Groups / Parameters	Sperm motility %	Sperm cell concentration x 10^3 / mm^3	Total abnormalities
Control	95.40 ± 1.30[a]	33.70 ± 1.25[a]	5.10 ± 0.55[a]
ACR low dose	56.10 ± 1.45[b]	11.30± 1.1.50[b]	10.60 ± 0.55[c]
ACR Medium dosed	40.15 ± 2.80[c]	7.50 ± 1.15 [c]	16.40 ± 1.65 [c]
ACR High dosed	26.40 ± 3.30 [c]	5.10 ± 0.60 [c]	34.45 ± 2.85 [c]
Chips group	50.90 ± 4.10 [c]	12.60 ± 1.20[b]	17.50 ± 1.65[b]

Means within the same column in each item carrying different superscripts are significantly difference at P≤0.05

Table (4): The effect of ACR on the mature male wister rats sex hormones and fertility.

Parameters / Groups	Testosterone µg/ml	FSH Ng/ml	Cortisol Ng/ml	LH mlu/ml	E_2 pg/ml	PRL ng/ml
Control	4.10±0.40 [a]	2.0±0.10 [a]	198±2.15 [a]	2.35±0.15[a]	44.5±1.15[a]	6.65±1.1[a]
ACR low dose	3.1±0.55 [b]	1.75±0.15[b]	175±1.6 [b]	3.4±0.25 [b]	49.6±0.9 [b]	8.6±1.25[b]
ACR Medium dosed	2.25±0.45 [b]	1.35±0.4[b]	162±1.5[b]	4.65±0.55[b]	55.5±0.65 [b]	13.3±1.45[b]
ACR High dosed	1.6±0.3 [c]	0.85±0.25[c]	144±1.15[c]	6.7±0.25[c]	67.5±1.55[c]	16±1.6 [c]
Chips group	2.9±0.15 [b]	1.8±0.3 [b]	170±1.7 [b]	3.55±0.2 [b]	48.6±1.25 [b]	8.2±1.15 [b]

(Mean ± SE) Means within the same column have different superscripts are significantly difference at P≤0.05 and highly significant (P≤ 0.01).

- FSH : Follicle stimulating hormone
- LH : Leutinizing hormone
- E2 : Stradiol
- PRH: Prolactin hormone

Table (5): Frequencies of chromosomal in mature male wister albino rat treated with different doses of acrylamide dosed orally and chips snack food every other day for 65 days .

Groups	Total aberrated cells	Chromosomal aberrations							
		Structural							Numerical
		Break	Gap	Sticky Chrom.	End to end association	Centeromiric attenuation	Ring chrom.	Chrom. Attenuations	pulverization
Control	1.8±0.55a	-	0.3±0.34[a]	0.9±0.45[a]	-	0.2±0.25[a]	0.4±0.15[a]	-	-
ACR low dose	30±3.60 [b]	5.6±0.55[b]	0.5±0.15[b]	4.4±0.57[b]	1.6±0.25[c]	1±0.36 [b]	0.5±0.2[b]	0.7± 0.35[b]	9.4±1.15[b]
ACR Medium dosed	38.9±2.95 [b]	8.9±1.65[b]	1.9±0.75[bc]	7.1±1.2 [b]	3.6±0.85[b]	1.5±0.4 [b]	0.9±0.15[b]	1.1±0.25[b]	5.5±1.15 [b]
ACR High dosed	76.8±5.7[c]	15.3±2.55[c]	3.2±0.36[c]	10.4±1.9[c]	7.5±0.6[c]	3.8±0.6[c]	2±0.25[c]	4.5±1.45[c]	13.6±2.45[c]
Chips group	1.9±0.44[a]	-	0.35±0.3[a]	0.85±0.4[a]	-	0.3±0.3[a]	0.4±0.15[a]	-	-

(Mean±SE) Means within the same column have different superscripts are significantly difference at P≤0.05 and highly significant (P≤0.01).

Table (6): The effect of ACR in treated and chips group every other day for 65 days on quantitative levels of both DNA & RNA in mature male wister rats testes.

Groups / Parameters	DNA mg/gm b.wt. testes	RNA mg/gm b.wt. testes
Control	30.65 ± 1.25 [a]	16.55 ± 0.35 [a]
Low dose ACR	22.10 ± 1.30 [b]	11.20 ± 1.15 [b]
Medium dose ACR	16.25 ± 1.35 [bc]	8.25 ± 0.75 [bc]
High dose ACR	12.15 ± 0.65 [c]	6.15 ± 1.50 [c]
Chips group	15.20 ± 1.20 [c]	5.20 ± 1.15 [c]

Means within the same column in each item carrying different superscripts are significantly difference at P≤0.05

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