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ACRYLONITRILE TESTICULAR SEMINOMA IN BEAGLE MALE DOGS (PATHOLOGICAL AND HORMONAL ASSAY)

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Abstract

Acrylonitrile (ACN) is highly poisonous compound with formula (CH3H3N) used in plastic solvent and acrylic fiber. This experiment was designed to evaluate the toxicopathological effect of ACN on male dog genitals.for acute (30) day period and chronic period (90)day. Twenty four healthy Beagle male dog aged e" 1 years were randomly divided in to three groups each contain each contain eight dogs : 1st (c) control group feeding for 90 day on normal dog diet, 2nd group (T_1) daily intake by stomach tube for 30 day ACN (30 mg/kg.b.w) with normal dog diet, while 3rd group (T_2) for (90) day daily intake by stomach tube ACN (30 mg/kg.b.w) with normal dog diet at days 30 and 90 hormonal assay of testosterone and FSH were Dane for all group with semen viability, concentration, PH and abnormality. Pathological changes on male genitalia (prostate, tastes and epididymis) were done on scarified dogs at day 90. T_1 and T_2 groups statistically significant decreased in testosterone level with increase of FSH hormone in serum mostly at T_2 in 90 day while T_2 showed statistically increased in semen PH, deformities while viability with concentration decreased. T_2 group indicated solid homogenous pale cut testis with microscopically lesion mostly at T_2 group characterized by polyhydral cells, distended seminiferous tubules and elongated palisading tumor with thick bands of partially hyalinized seminoma.

Introduction

Acrylonitrile it is an highly poisonous compound with the formula (C_3H_3N), colorless, volatile liquid in a characteristic odor with boiling point (77.3c), onion or garlic – like odor, although commercial sample can be yellow due to impurities, Also acrylonitrile (CH2=CH-C=N) is soluble in water and miscible with most organic solvent, Technical –grade acrylonitrile is more than 99% pure, with minor quantities of impurities and stabilizer (Abdl-El Azeim *et al.,* 2012). Acrylonitrile is used mostly to make plastics, acrylic fibers, and synthetic rubber. Because acrylonitrile evaporates quickly, it is the most likely to be found in the air around chemical plants where it is made. Acrylonitrile break down quickly in the air, it has been found in small amount in the water and soil near manufacturing plants and hazardous waste sites (Simon *et al.,* 2012*;* Lee *et al.,* 2015). ACN is an irritant compound causing sever irritant with many tissue and toxic in all routs and observed readily causes systemic toxicity (IPCS, 1983). Testicular seminoma is a tumor of younger males affected functional and active young males (Tan *et al.,* 2011*;* COST, 2013). Incidence might be as high as 11.5 per 100000 in white men compared with 1-2 per 100000 in negrow (Bray *et al.,* 2006).

Seminoma side effects can include lung injury. Metabolic syndrome, renal toxicity and decrease fertility, testicular cancer will alive for decades (Brain and Siamak, 2018), its testicular germ cell tumor originate in germ cell (seminoma and non- seminomas) sometimes combination of these two groups and more 70% of cases are diagnostic germ cell tumor recorded over the second half of the 20th century in all ages, congenital malformation of male genitalie is strong risk factor with germ cell tumor (Stamp and Jacobsen, 1995*;* Sobin and Withekind, 2002). The most recent who histologic classification of testis tumor is similar to 1998 classification and differ from teratoma

and polyembryomas (Mostifi and Sesterhenn, 1998*;* Eble *et al.,* 2004) who classification 2016 germ cell tumors and broadly classified into two categories, germ cell tumor derived for germ cell neoplasia in situ and germ cell tumor unrelated to germ cell neoplasia in situ, the former category is further divided into four categories consisting noninvasive germ cells neoplasia tumors of single histological type, non-seminomatous germ cell tumor of more than one histological type, germ cell of unknown types, seminoma is major tumor of single histological types and account for 5% of testicular tumor. its differentiate along the gonadal cell lines and resemble primordial germ cells and genocyte. Non-seminomatous germ cell tumor crNSGC in turn have retained pluripotency and include a number of different subgroups, mostly seminoma cause higher follicle stimulating hormone (FSH) and lower testosterone level in serum (Sumat *et al.,* 2017). There is no information on ACN toxicity and development on male reproductive system (CCOHS, 2000). We designed the experiment to establish the toxicopathological effect of CAN on testicular seminoma in male Beagle dogs

Materials and Methods

Twenty four (24) healthy Beagle dogs ($\geq$ 1 year) of selected to participate in our study. The food formulated to meat or exceed nutrient recommendation according to American feed central officials (AAFCO, 2000) and management in animal house of veterinary collage, Baghdad university. Baghdad, Iraq. Under 12 hour light and 12 hour dark at 20±2 C° for 90 days. All experiment animal groups under identical management protocol. All hormonal and pathological analysis were done in department of pathology, College of veterinary medicine, University of Baghdad Baghdad Iraq.

Toxicant of male dogs

Male dogs groups were randomly divided into three equal group : control group (c) contain 8 male dogs feed on normal dogs diet (AAFCO, 2000), 2nd group (T1) contain 8 male dogs administrated daily by stomach tube (ST) in drinking water for 30 days (acute dose) ACN with feed on normal dog diet, 3rd group contain 8 male dogs administrated daily by stomach tube in drinking water for 90 days (chronic dose) ACN with feed on normal dog diet. ACN is obtained from sigma compound / Germany at dose (40mg / kg BW) (IPCS, 2002). All animal were sacrificed for postmortem examination, blood samples were collected from jugular vein in test tubes to measured the level of testosterone and FSH hormone. Semen collected and evaluated (analysis) from the tail of epididymis, hormone and semen analysis were done at day 30 and 90 while pathological analysis at day 90 of experiment.

Hormonal assay

Blood sample were collected at day 30 and 90 from animal group from jugular vein for determination of serum testosterone and FSH concentration.

A) Testosterone analysis by Elisa

Format the microplate wells for each serum reference, control and treated then 10µl of the appropriate serum reference or specimen into each well after adding 50 µl of working testosterone enzyme reagent to all wells. Than swirl the microplate 20-30 second and adding 50 µl of testosterone biotin reagent with swirl the microplate 20-30 second and incubation 60 minutes at room temperature with discard the contents of the microplate, for three times adding 300 µl of wash buffer, 100 µl of working substrate solution into well with incubation 15 minute at room temperature then add 50 µl stop solution to each and swirl the microplate 20-30 second, then read the absorbance in each wells at 450nm.

B) FSH hormonal assay

After blood sample collection and put it in sample tube then in Eppendorf tube and centrifuged at 3000 rpm for 15 min. the supernatant was transferred to a (1.5 ml) Eppendorf tube and stored at -20 C° FSH were measured using Radio-immune assay technique. All the samples measured at same time to minimize the error (Caligioni, 2009).

Semen analysis

Sperms from all dogs groups were collected from epididymis tail by slicing and rinsing pressing techniques of each epididymis in 5 ml of physiological normal saline at 37 C° in small glass dish as fallowing (Tajik and Hassan, 2008).

a. Sperm viability (Dead and live) (Turk *et al.*, 2007)

On glass slide a drop from sperm suspension with a drop of Nigrosine-Eosin stain mixed together. From each sample two smears were done and dry then covered by cover slip. In each smear 200 sperm under light microscope at x40 objective power were examined, finally percentage were calculated after taken average of two smear :

Sperm viability% =Number sperm alive / total number of sperm x 100

b. Semen PH : (Carr *et al.*, 1985)

Semen PH were done immediately after collection by PH meter.

c. Individual motility (Tejerini *et al.*, 2009)

On a warm glass slide a drop of sperm suspension

placed at 37 C° were covered by cover slides and under objective x40 light microscope examined, only progressive forward spermatozoa a movement other movement were missed.

d. Sperm abnormalities : (Hatif and Abood, 2011)

On the edge of glass slide a drop of sperm suspension placed and then a drop of Nigrosine- Eosin stain to 3 drop of tryban blue stain mixed with 2 smear of this prepared on other glass slide were done for abnormal sperm morphology and on light microscope and fluorescent microscope for tryban blue stain under x40 and x100 power examination. 200-400 sperm calculated and then average were taken.

Sperm abnormalities% = number of abnormal sperm/ total number of sperm x 100.

Pathological analysis

At the end of experiment (90) day animals were sacrified by injection of highly dose of ketamine hydrochloride intramuscular. Grossly appearances were recorded to detect any abnormal lesion in the male genital system. Tastes, prostate gland and epididmis specimen were taken from animals and kept in 10% formaldehyde solution for fixation and then processed routinely by using histokinate. Tissue section were embedded in paraffin and sectioned by microtome with hematoxylin and Eosin stained. The examination done under light microscope (Lee and Luna, 1968).

Data analysis

Standard error and Standard deviation was used to compare the significant difference between means. Statistical analysis system was used to analyze Data (SAS, 2010).

Results

a. Testosterone analysis :

Table 1: ACN toxicity in male dogs testosterone hormone concentration (mg/dl).

Days / Groups	30 days	60 day	p
Control (G1)	2.26±0.003	2.26± 0.009	p < 0.0001
Treated 1(G2)	81±0.002	38± 0.066	p < 0.0001
Treated 2(G2)	81±0.002	31± 0.003	p < 0.0001

b. Semen viability :

c. Semen PH :

d. Sperm counting (X1000000/ml)

e. Sperm deformities :

Serum testosterone level showed significant increase

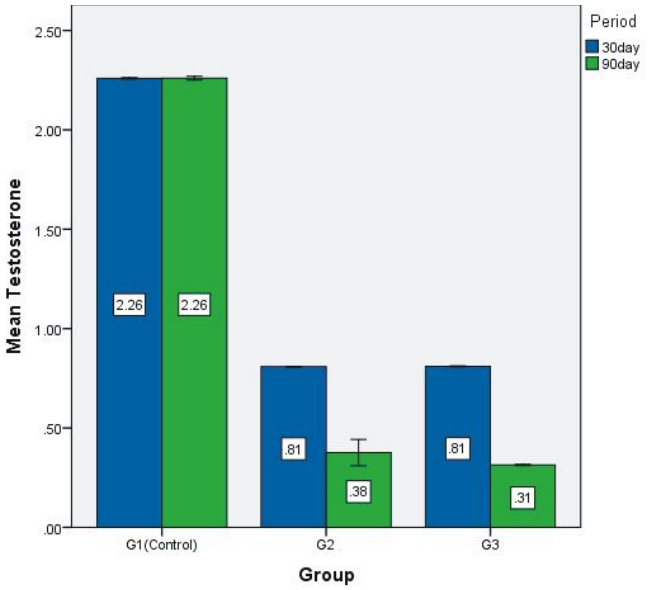


Fig. 1: ACN toxicity in male dogs testosterone hormone concentration (mg/dl).

Table 2: ACN toxicity in male dogs FSH hormone (ng/ml).

Days / Groups	30 days	60 day	p
Control (G1)	81 ± 0.004	81± 0.004	p < 0.0001
Treated 1(G2)	1.64±0.013	1.77± 0.009	p < 0.0001
Treated 2(G2)	1.63± 0.009	1.90 ± 0.006	p < 0.0001

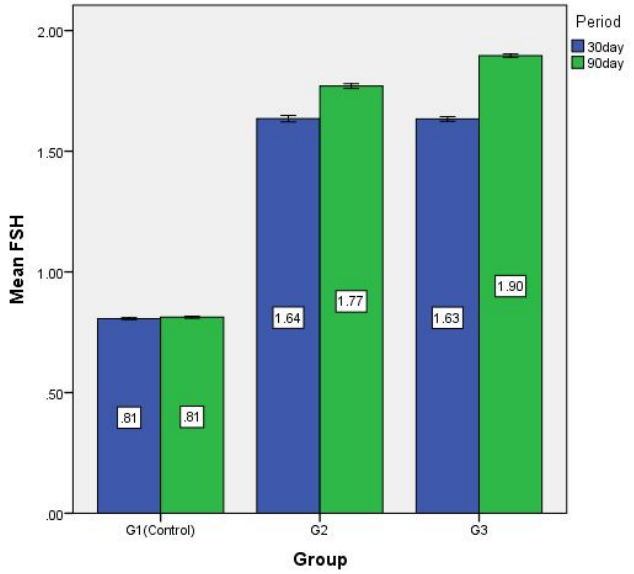


Fig. 2: ACN toxicity in male dogs FSH hormone (ng/ml).

in T_1 and T_2 groups especially at T_2 group in 90 days compare with control group.while FSH hormone indicated significant increase in the level of FSH at T_2 group mostly at day 90 (1.90 ± 0.006) compare with control group and T_1 at day 30. Semen quality (viability, motility, sperm count, and deformities) indicated significant decrease at T_2 group mostly at day 90 compare with T_1 and Control

Table 3: ACN toxicity in male dogs on semen viability (alive and dead).

Groups \ Days	30 days	60 day	p
Control (G1)	61.63± 0.38	60.50± 0.33	p < 0.0001
Treated 1(G2)	47.38±0.84	48.50±0.71	p < 0.0001
Treated 2(G2)	40.75± 0.31	32.25± 0.82	p < 0.0001

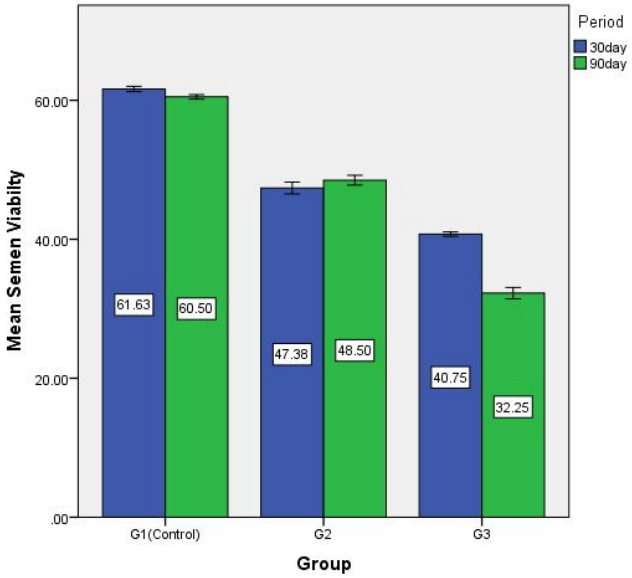


Fig. 3: ACN toxicity in male dogs on semen viability (alive and dead).

Table 4: ACN toxicity in male dogs on semen PH.

Groups \ Days	30 days	60 day	p
Control (G1)	913± 0.029	963± 0.026	p < 0.0001
Treated 1(G2)	3.875±0.125	4.50± 0.189	p < 0.0001
Treated 2(G2)	8.50± 0.267	8.625± 0.263	p < 0.0001

group (32.25± 0.82, 48.50±0.71, and 60.50 ± 0.33) Respectively while PH of semen decreased significant difference mostly at day 30 and 90 when compare with control group (at day 30 3.875 ± 0.125, 8.50 ± 0.267 at day 60 4.50 ±0.189, 8.625±0.263 and control group (913± 0.029, 963±0.026).

Pathological changes

No important pathological changes (grossly and microscopically) observed in the control group figure (7,A) at day 30 and 90.

Grossly appearance of testes is more sever at T_2 group than T_1, the cut surface of seminoma is white and grayish color, bulging and glistening, in some section homogeneous pale surface.

Microscopic group

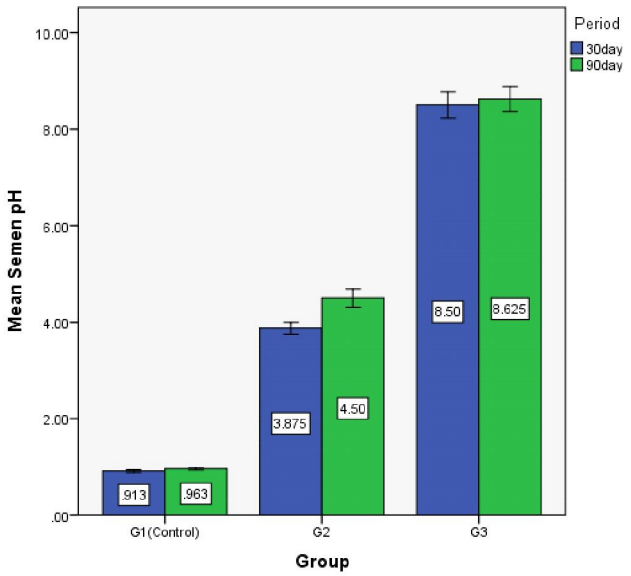


Fig. 4: ACN toxicity in male dogs on semen PH.

Table 5: ACN toxicity on male dogs sperm counting (X1000000/ml).

Groups \ Days	30 days	60 day	p
Control (G1)	5467.50± 12.356	5504.13±25.204	p < 0.0001
Treated 1(G2)	4321.25±19.769	4191.88± 13.294	p < 0.0001
Treated 2(G2)	4255.38±14.316	2053.50±18.615	p < 0.0001

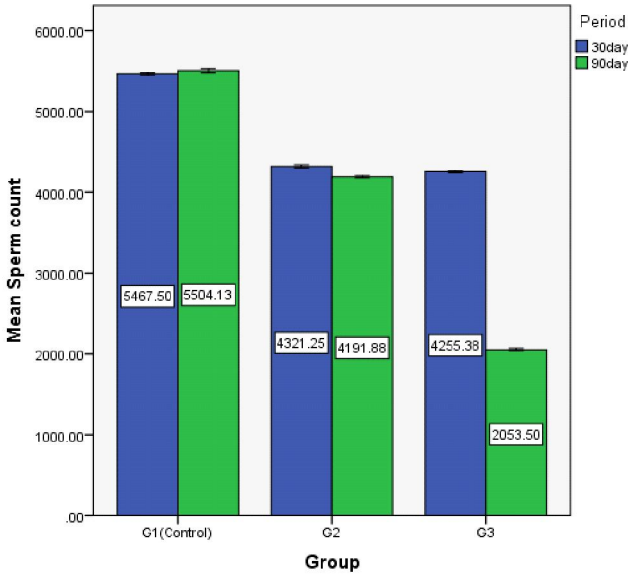


Fig. 5: ACN toxicity on male dogs sperm counting (X1000000/ml).

T_1 group: the epididymis architecture showed no lear microscopic alteration, prostate gland showed alveolar damage due to epithelial cells sloughing with alveolar deformities (elongated) in some area cystic alveolar forms with increase hyperplasia of alveolar epithelium more than 3-5 layer cuboidal to columnar cells. Mononuclear cells infiltration in the stromal tissue mostly macrophage and

Table 6: ACN toxicity on male dogs sperm deformities %.

Groups \ Days	30 days	60 day	p
Control (G1)	11.13± 0.639	10.50±0.327	p < 0.0001
Treated 1(G2)	22.38±0.822	41.50±0.627	p < 0.0001
Treated 2(G2)	22.50± 0.707	62.00±0.824	p < 0.0001

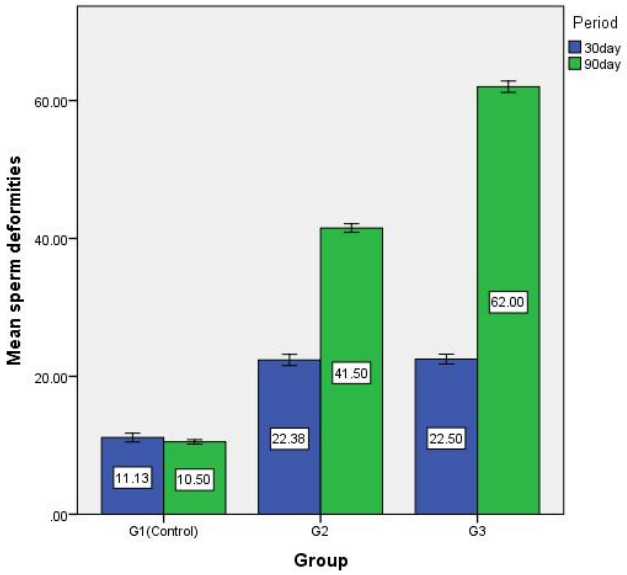
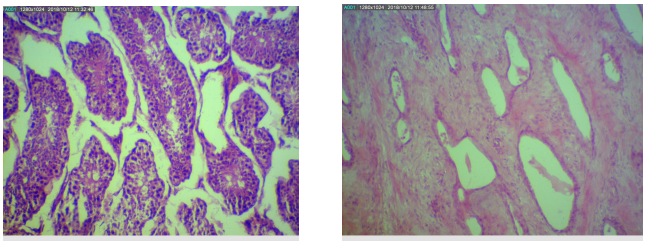


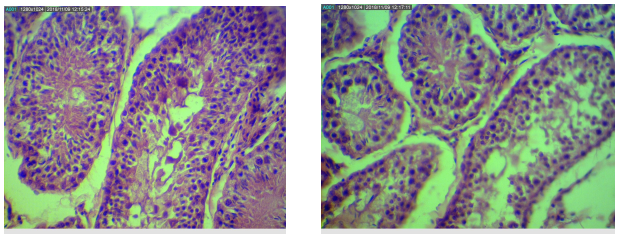
Fig. 6: ACN toxicity on male dogs sperm deformities %.

plasma cells Fig. (7, B). Testis showed nuclear pyknosis of necrotic cells and many multinucleated gaint cells in the lumen of seminiferous tubules formed by fusion of degenerated spermatogenic cells fig (8 A,B,9 A,B). T_2 ACN administrated dog showed that prostate gland sever cystic dilatation and sloughing of alveoli with hyperplasia of alveoli epithelium look like papillary and glandular projection with sever stromal mononuclear cells infiltration mostly macrophages Fig. (10 A, B) testis were revealed diffuse hyperplasia characterized by tall epithelium and papillary growth into the lumen some cystic gland as well as broad sheets of hyperplastic fibromuscular tissue with highly mitotic Fig. (11 A, B,12,A).in other section alveolar papillary type adenocarcinoma characterized by papillary growth of epithelium into alveoli like space surrounded by bands of connective tissue, the space are almost completely filled with tumor cells (polyhedral shape) and necrosis Fig. (12 B, 13 A). Multi mass of tumor irregular in shape replacing the seminiferous tubules (seminoma) composed of large polyhydral cells with prominent nucleolus assembling spermatogenic cells with mononuclear cells accumulation. Some times stented seminiferous tubules with solid elongated and palisading tumor cells having pale cytoplasm separated by thick bands of partially hyalinized fibrous tissue (13 B, 14 A, B, C).



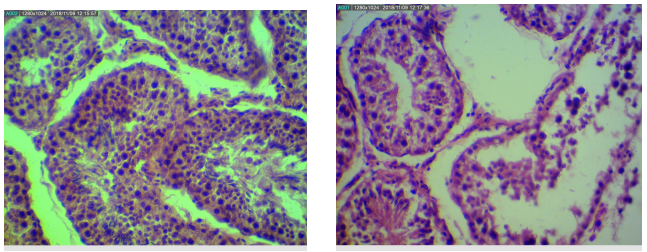
(A) (B)

Fig. (7: A, B): Histopathological section of (A) testis of group C showed normal architecture testis (H and E x40) (B) prostate gland of T_1 group administrated ACN for 30 day (black arrow) alveolar damage with epithelial sloughing (blue arrow) alveolar deformities (red arrow) hyperplasia epithelium (yellow arrow) mononuclear cells infiltration (H and E x40) .



(A) (B)

Fig. (8: A, B): Histopathological section of testis of T_1 group administrated ACN for 30 days showed (black arrow) nuclear necrosis (blue arrow) multinucleated giant cells in lumen (spermatogenic cells) (red arrow) vacuolation seminiferous epithelium (yellow arrow) complete necrosis of tubules (H and E x40) .

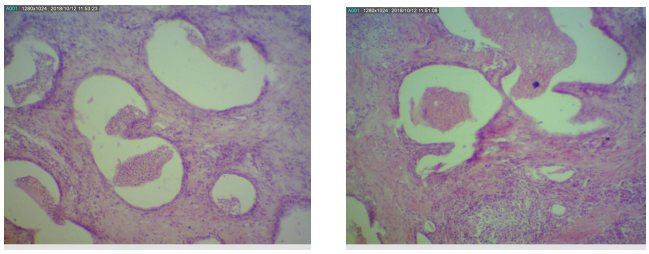


(A) (B)

Fig. (9: A, B): Histopathological section of testis of T_1 group administrated ACN for 30 days showed (black arrow) nuclear necrosis (blue arrow) multinucleated giant cells in lumen (spermatogenic cells) (red arrow) vacuolation seminiferous epithelium (yellow arrow) complete necrosis of tubules (H and E x40) .

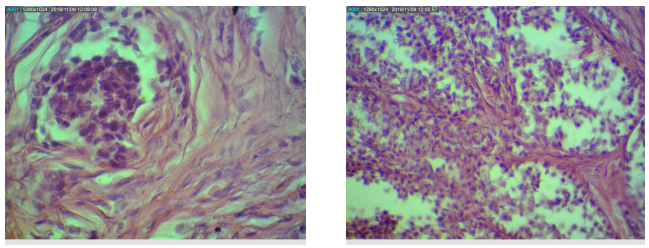
Discussion

There is no information on ACN toxicity in human and animal and development on reproductive system (CCOHS, 2000). Serum testosterone level decrease mostly at chronic period with T_2 group may either due to effect of ACN on the pathways androgen biosynthesis, or its toxic effect on brain hypothalamus anteriopituitary gland due to one of the metabolic effects of ACN is



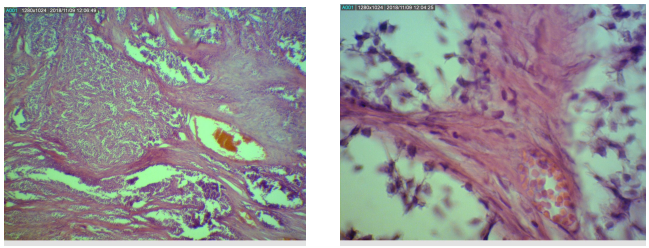
(A) (B)

Fig. (10: A, B): Histopathological section of prostate gland of T_2 group administrated ACN for 90 days showed (black arrow) sever cystic dilation and deformities alveolar cyst (blue arrow) alveoli sloughing epithelium (red arrow) hyperplasia epithelium like glandular or papillary projection (yellow arrow) stromal mononuclear cells infiltration (white arrow) macrophage granuloma (H and E x40) .



(A) (B)

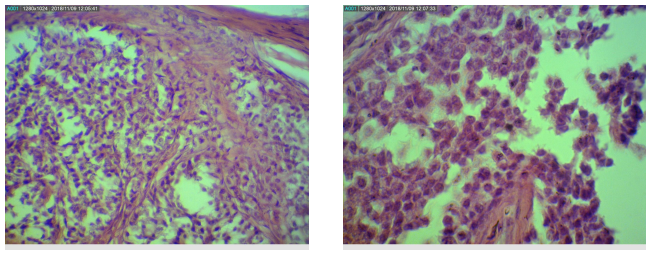
Fig. (11: A, B): Histopathological section of testis of T_2 group administrated ACN for 90 days showed (black arrow) diffuse hyperplasia with papillary growth (blue arrow) broad hyperplasia fibromuscular tissue (red arrow) mitotic figure (H and E x40) .



(A) (B)

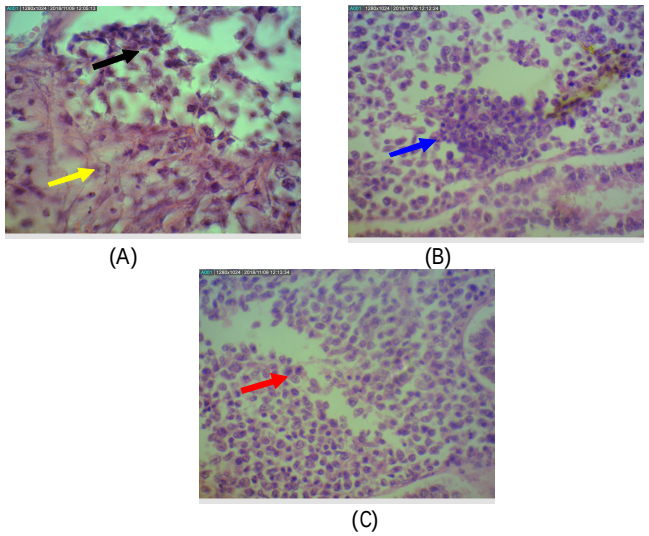
Fig. (12: A, B): Histopathological section of testis of T_2 group administrated ACN for 90 days showed (A) (black arrow) diffuse hyperplasia with papillary growth (blue arrow) broad hyperplasia fibromuscular tissue (red arrow) mitotic figure (B) (black arrow) papillary adenocarcinoma (blue arrow) bands of connective tissue (red arrow) tumor cells polyhydral in shape (H and E x40) .

Cyanide toxicity on central neuron system (HSE, 2005) and long term exposure to ACN cause C.N.S depression (ATSDR, 1990). FSH hormone concentration level showed increased significantly at T_2 in 90 day due to ACN is trigger effects on dopamine and induced noradrenaline turnover in various parts of hypothalamus, several hormones in hypothalamus inducing FSH



(A) (B)

Fig. (13: A, B): Histopathological section of testis of T_2 group administrated ACN for 90 days showed (A) (black arrow) diffuse hyperplasia with papillary growth (blue arrow) broad hyperplasia fibromuscular tissue (red arrow) mitotic figure (B) (black arrow) papillary adenocarcinoma (blue arrow) bands of connective tissue (red arrow) tumor cells polyhydral in shape. (H and E x40) .



(A) (B) (C)

Fig. (14: A, B, C): Histopathological section of testis of T_2 group administrated ACN for 90 days showed (black arrow) polyhydral prominent nucleus cells (blue arrow) palisading tumor cells (red arrow) pale cytoplasm (yellow arrow) partially hyalinized fibrous tissue (H and E x40) .

controlled and regulated by dopaminergic system, so the cyanide toxicity of ACN cause increase secretion of FSH hormone. ACN is readily absorbed into systemic circulation, following ingestion, inhalation or dermal exposure and when undergo metabolism either by glutathione conjugation which yields 2-cyanoethylmercaptue acid or cytochrome 450 oxidation with yield 2-cyanoethylene oxide metabolized into cyanide and cyanide is undergo depression effects on brain (Wakefield, 2007). The pathway of ACN metabolism is important spreads oxidation by cytochrome p450 2E1 forms 2-cyanoethlene oxide. Sperm deformation results from unscheduled DNA synthesis and mutagenicity of ACN on sperm formation (HSE, 2005). Research indicated that ACN shown reverse mutation in gene TA1535 and TA100 strain of salmonella typhimurium and

in hamster or rat 59 microsomal fractions (HSE, 2005).

ACN is carcinogenic to laboratory animals and workers to produce pulmonary cancer which classified as (group 2B) cancer (IARC) and under EU system as a category 2 carcinogen (ATSDR, 1990) seminoma observed mostly at T_2 group in 90 day mostly due to CAN toxicity, most tumor of testis are germ cells origin and like the totipotent germ cells from where they arise, ACN is readily absorbed into circulation system specially after ingestion and metabolism into cyanide causing symptom and pathological lesion, so systemic toxicity at the site of contact with incidence of genotoxicity (chromosomal aberration) specially in workers occupationally exposed to ACN. The carcinogenicity mostly clastogenicity in bone marrow (metaphase analysis of micronucleus). Recent studies have found increased of seminoma in metal worker, leather worker, unionized carpenter, paper mill maintenance employees and writer due to toxicity of compound and oxidative stress of ACN on living tissue which indicated the cancer, so ACN is considered to be possible human carcinogen (Wakefield, 2007).

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