

## **Effect of dietary omega 3 essential polyunsaturated fatty acid on reproductive system of male rats that exposed to acrylamide**

**تأثير إضافة الحامض الدهني المشبع الأساسي الأوميغا 3 على الجهاز التناسلي الذكري للجرذان المعرضة للاكرالاميد**

Shaima Amore Jabbar/ assistance lecture/ Pharmacy College/ Karbala University/ Msc.

Physiology

Email: shaimaamore@yahoo.com

Morug Salih Mahde/ Lecturer/ pharmacy College/ Karbala University/ Dr. Clinical

Biochemistry

Email: [morug\\_ua2008@yahoo.com](mailto:morug_ua2008@yahoo.com)

### **Abstract**

Acrylamide(ACR) has a range of toxicological hazards including reproductive toxicity, So in this research we studied the effect of acrylamide on male reproductive system of rat that drenched with omega 3 poly unsaturated fatty acids at the same time. We used 40 laboratory male rats provided by AL- Nahrain infertility center, They were housed in plastic cages in animal house at Karbala University/ Education College, however these animals divided randomly in to four group( group 1 control, group 2 drenched with acrylamide10% and omega 3 according to body weight, group 3 treated with omega 3 only according to body weight and group 4 drenched with acrylamide10% only ). The results showed that there are significant decreases  $P \leq 0.01$  in testosterone hormone in forth group as compared with other groups in the same time there are a significant increase  $P \leq 0.01$  in third group as compared with others, while the pathological changes in testis slide section in forth group demonstrated un recover degeneration (necrosis) and acute inflammation characterized by present of inflammatory cells in stroma and seminiferous tubules as compared with other groups.

### **الخلاصة:**

يعتبر الاكرالاميد من المواد الكيميائية ذات التأثير السمي الخطر على الجهاز التناسلي . استخدمت في هذه الدراسة اربعون حيوان من ذكور الجرذان البيضاءحصل عليها من مركز جامعة النهرين للعقم بعمر 60 يوم وقسمت بطريقة عشوائية إلى أربعة مجاميع : واعتبرت المجموعة الاولى مجموعة سيطرة في حين غذيت المجموعة الثانية على الاكرالاميد 10% والاوميغا3 محسوبة الجرع حسب وزن الجسم في حين جرعت المجموعة الثالثة بالاوميغا 3 فقط محسوبة الجرع حسب وزن بينما عوملت المجموعة الرابعة بالاكرالاميد فقط بتركيز 10% وأظهرت النتائج زيادة معنوية عند مستوى مقارنة ( $p < 0.01$ ) في مستوى هرمون التستستيرون للمجموعة الثالثة عند مقارنتها مع المجاميع الأخرى في حين سجلت المجموعة الرابعة انخفاض معنوي ( $p < 0.01$ ) في مستوى هرمون الاستروجين بين المجموعة الثانية عند مقارنتها مع مجموعة السيطرة. وفي الحين ذاته أظهرت المقاطع النسيجية للخصية للمجموعة الرابعة تلف نسيجي واضح لايمكن شفائه بإزالة المسبب وإضافة إلى تشخيص التهاب نسيجي حاد يتميز بارتشاح الخلايا الالتهابية.

**Introduction:**

Sex is important for continuous animals life, so some people eat to life while other life to eat, but scientists devoted their efforts to draw a typical map of what is important to eat which consider safe to our life.

Acrylamide (  $C_3H_5HNO$  ) is a small hydrophilic molecule that polymerized readily in the presence of an initiator because of the double bond between the first and second C-atoms, which makes it a versatile industrial chemical(1,2).

Before 2002, acrylamide exposure was thought to occur mainly through occupational exposure, also although through the consumption of water and the use of cosmetics(3). However it has recently been shown that acrylamide forms when foods are cooked at normal cooking temperatures (e.g., frying, grilling and baking). Sharp (2003) and others reported that carbohydrate-rich foods, when heated above approximately 248°F (120C°), had acrylamide concentrations up to 1mg/kg food (4). There are several studies on deferent species of laboratory animals such as cats, rats, mice, guinea pigs, rabbits, and Monkeys(5), found that repeated daily exposure at levels of 0.5–50 mg ACR/kg/day result in a triad of effects such as hind-limb foot splay, ataxia, and skeletal muscle weakness as measured by decreased fore- and hind-limb grip strength. The neurotoxic effects of ACR in humans in occupational settings were documented (6,7). As noted earlier, neurotoxicity was recently observed in construction workers using a waterproofing sealing gel that contained ACR(8). The clinical signs were of peripheral neuropathy, which manifested as tingling and numbness of the hands and feet, weak legs, and loss of toe reflexes, all of which were reversible (9). Longer exposures resulted in cerebellar dysfunction, excessive tiredness, ataxia, and some central neuropathy, which was also reversible in most cases (9).

Omega-3 ( poly unsaturated fatty acids) such as eicosapentaenoic acid (EPA) and docosahexanoic acid (DHA) found in fish. The reasons why these fatty substances are so important revolve around their role in cellular membranes. A diet that is deficient in omega-3 fatty acids, particularly EPA and DHA, results in altered cell membranes. Without a healthy membrane, cells lose their ability to hold water, vital nutrients, and electrolytes. They also lose their ability to communicate with other cells and be controlled by regulating hormones. They simply do not function properly. Cell membrane dysfunction is a critical factor in the development of virtually every chronic disease, especially cancer, diabetes, arthritis, and heart disease. Not surprisingly, long-chain omega-3 fatty acids have shown tremendous protective effects against all of these diseases (10,11,12 ).

From the above we concluded that both acrylamide and omega 3 are present in diet, but first one occur in ways out of our desire, but the second can be chosen by ourselves, so the aims from this experiment to study the effect of dangerous materials like acrylamide present in our food even in trace amount with the effect of essential nutrition like omega 3, not synthesis in human body, but he could get it from diet.

However we focus on rat male reproductive to importance of sexuality in animal lives, so we study the effect of omega 3 and acrylamide on testosterone hormone, because is an androgens are often called “male hormones. Testosterone is made in the adrenal glands and ovaries. It is important to measure both free and bound testosterone since only about 1% is free, the rest is bound to SHBG (sex hormone binding globulin) which carries the testosterone in blood. Testosterone has a myriad of functions in the human body ( motivation, emotional well being, self-confidence, increases muscle mass and strength, increases sexual interest, helps maintain memory, helps maintain bone strength, decreases excessive body fat, increases muscle tone so your skin does not sag( 13,14 ).

In the other hand we studied the histological changes in testis by making special histological section.

**Materials and methods:**

**Animals and experimental protocol:**

Fourteen adult laboratory male rats (100-150g), aged about 70 days, provided by AL- Nahrain infertility center, they were housed in plastic cages in animal house at Karbala University/ Education College, so it's placed on a 12-hour light/dark cycle, with food and water freely available. Rats were allowed at least 1 week to adapt to the experiment environment.

The dosing solutions were prepared every week by mixing acrylamide with saline W/V, 10%. Dosing solutions were stored at 4°C for no longer than 1 week prior to use. Drugs were exposed by oral gavages in a total volume of 0.2 mL four times a week for 12 consecutive weeks (15, 16, and 17).

Omega 3 fatty acids provided commercially by(2119 S. Wilson Street, Tempe, AZ 85282 USA. 21<sup>st</sup> century laboratories, INCt), fatty acids were exposed by oral gavages daily, in which doses calculated according to body weight.

**Animals design:**

Rats were randomly distributed into four different groups/ten animals for each one, however these groups named G1( control ) and G2( treatment with acrylamide 10% and omega 3 according to body weight ), G3 ( drenched with omega 3 only according to body weight) and G4 ( drenched with acrylamide 10% only ).

**Preparation of testis slides:**

Rats were anesthetized with ether then animals were scarified and testes are taken off and kept in ice to keep tissues shape from changing for 10 minutes, after that testes immersed into 4% polyformaldehyde for 24 hours, dehydrated in 50%–100% ethanol, washed in xylene and embedded in paraffin wax. The central cross-cut serial sections 6 µm thick were obtained every 3 mm of the tissue and then were subject to a hemotoxylin and eosin stain (18).

**Biochemical assay of plasma samples for testosterone hormone:**

Blood samples were collected after 12 weeks by heart puncture in anesthetic animals with methohexital anesthesia 60mg/kg, administrated intaperitoneally(19) according to ethical guidelines. However blood collected in EDTA tubes then centrifuged for 10 minutes with 10000 rpm to collect plasma and kept in frozen at -20° C until assay. So concentration of testosterone hormone were determined in plasma by commercial available enzyme immunoassay kits ( IBL GESELLSCHAFT FUR IMMUNBIOLOGIE MBH, Hamburg- Germany ).

**Statistical analysis:**

Data were expressed as mean ± SD. Differences between control and other groups were tested for significances using a one - way analysis of variances (ANOVA). P- Values of 0.05 or less were considered significant, statistical analyses were performed using SPSS for windows version (SPSS, Inc., Chicago, Illinois).

**Results:**

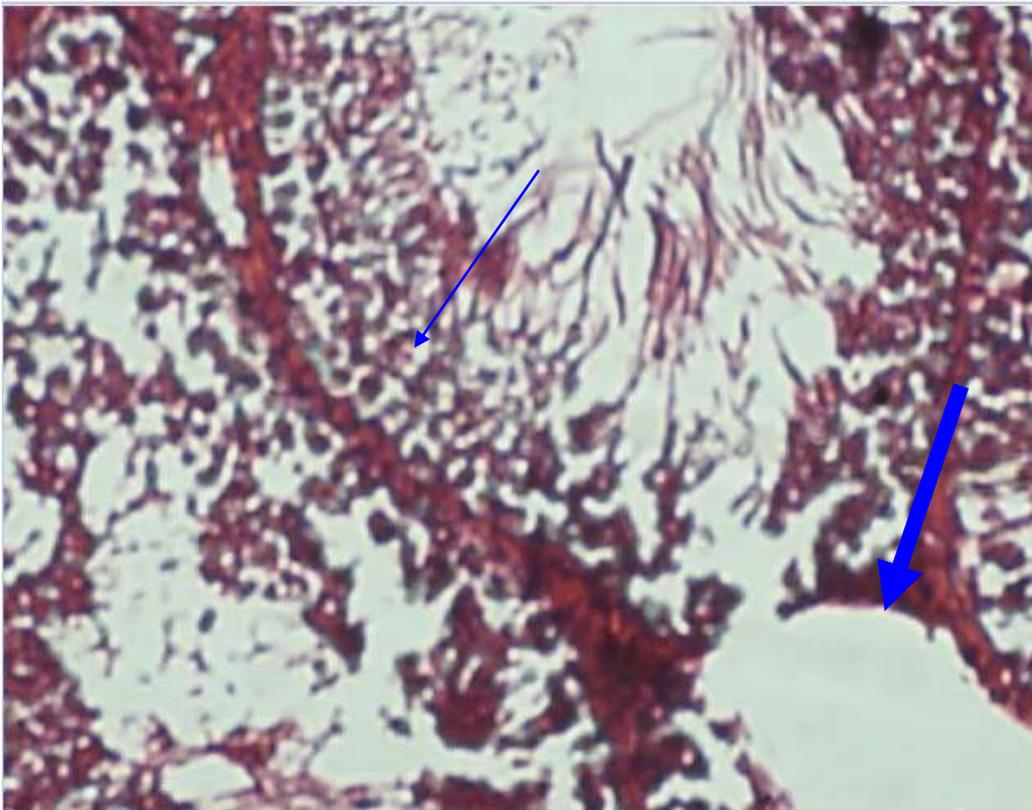
Table ( 1 ) summarizes the mean value ± SD of plasma testosterone in ng/ml, there was a significant decrease  $p < 0.01$  in plasma testosterone hormone in treated group with acrylamide only as compared with G1,G3 and G2, while there are no significant changes between group drenched with omega 3 and acrylamide as compared with control in  $P < 0.01$  in the other hand there was a significant increase in plasma testosterone hormone in group treated with omega 3 fatty acids only as compared with the other groups

Table (1) summarizes plasma testosterone hormone levels in mal rat in ng/ml.

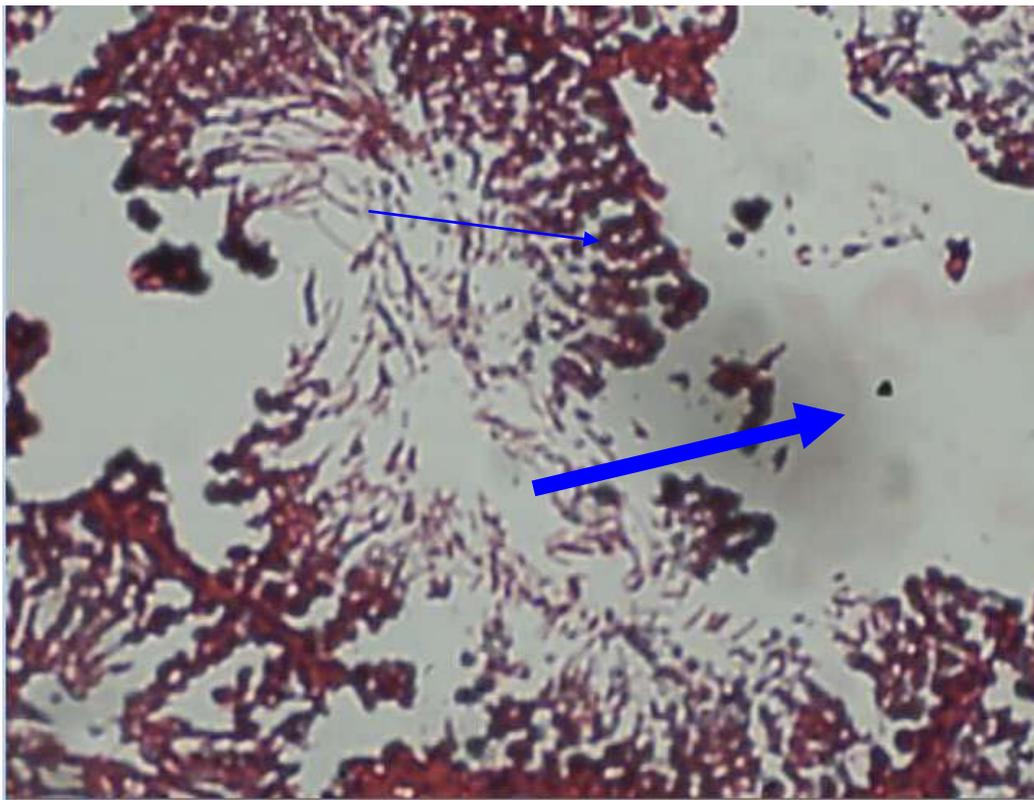
Group name	Testosterone ng/ml.
Control G1	3.704 $\pm$ 0.020 A
Acrylamide and omega 3 G2	3.684 $\pm$ 0.1.097 A
Omega 3 only G3	4.182 $\pm$ 0.478 B
Acrylamide only G4	2.607 $\pm$ 1.077 C

- Same capital letter mean no significant changing
- Different capital letter mean significant changing
- $P \leq 0.01$

In the other hand there are several histological changes in testes slide section in which we can summarize as below:

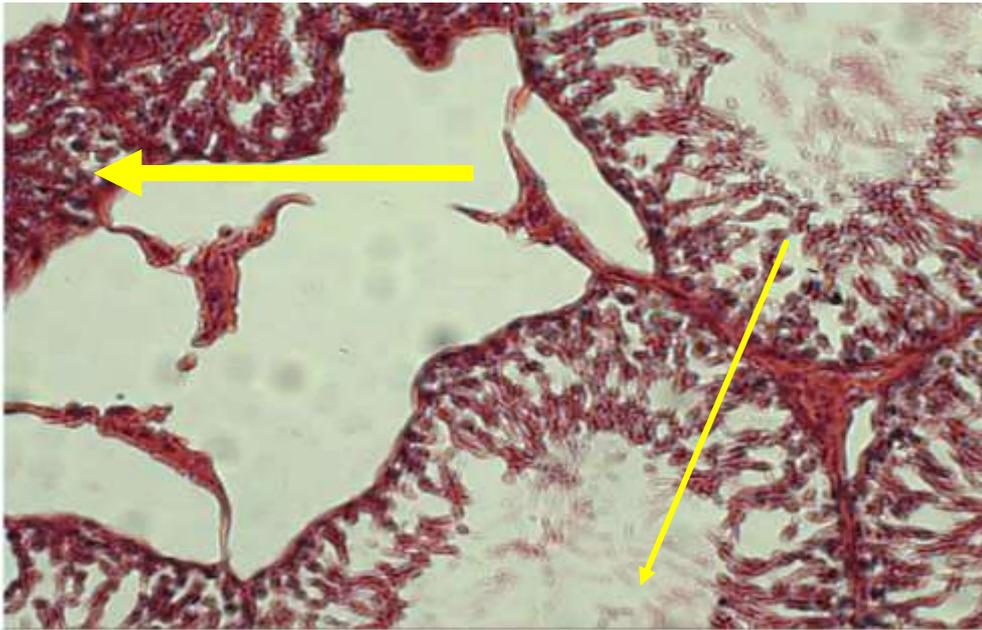


**Picture (1) testis section of male rat (group treated with acrylamide 10% only) X10**

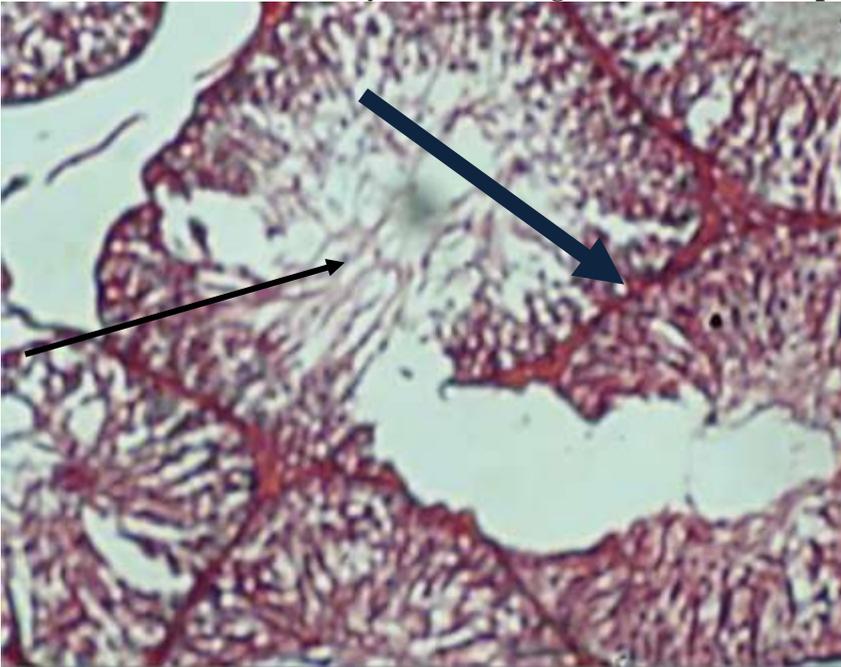


**Picture (2) testis section of male rat ( group treated with acrylamide 10% only) X10**

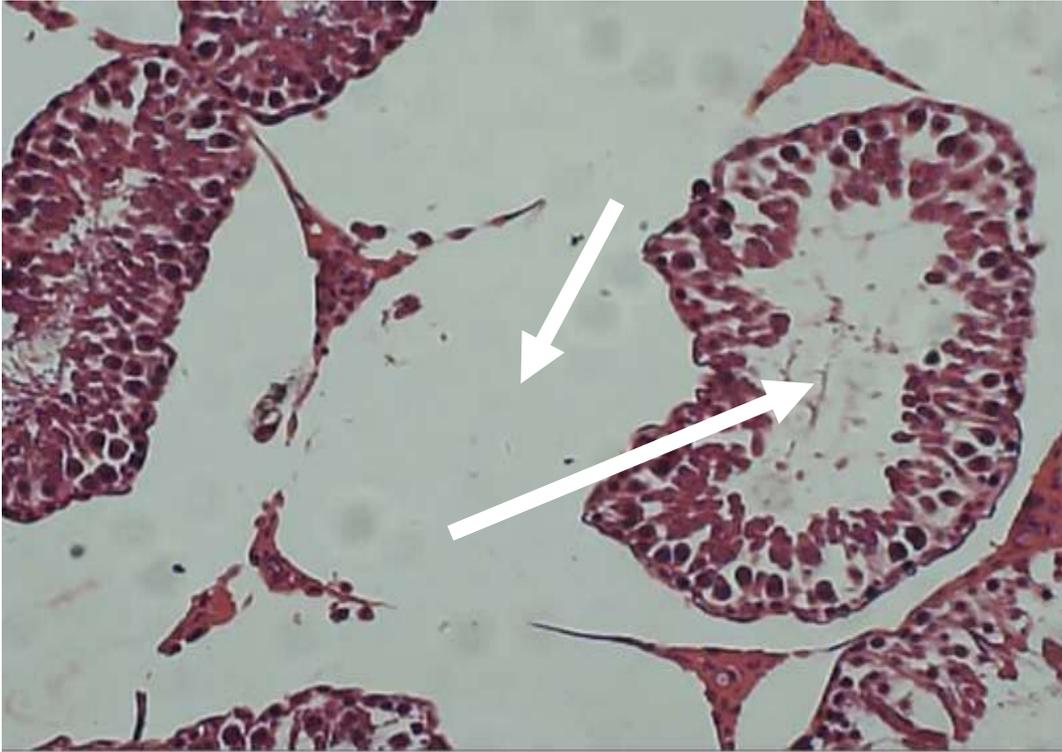
In pictures (1and2) of testis sections for group treated with acrylamide10% can be summarize from the cells normally present in the lumen of the tubule have been completely destroyed ( thin blue arrow) with occasional of necrosis areas ( thick blue arrow ). In the other hand we can illustrated by infiltration of chronic inflammatory cells in the stroma ( lymphocytes and plasma cells ) in picture 3 ( yellow thick arrow ) with degeneration of new sperm ( yellow thin arrow ) in addition there is a destruction of stroma and accumulation of edema and hemosedrine in center of somniferous( thick and thin black arrow) in picture 4 and there is necrosis area which represented by white thick arrow in picture 5. In the other hand there is no necrosis area in histological section picture of male rat in group drenched with acrylamide and omega 3 fatty acids, but we can be manifestation simple degeneration of spermatic cyst and mild hemosidrine (thin red arrow in picture 6), while we can see normal stroma and normal spermatogenesis in picture 7 represented by thick white arrow. Simultaneously we didn't record pathological changes in testis section of group drenched with omega 3 only, so there were normal stroma connective tissue and normal spermatogenesis can be illustrated in picture 8 and 9 by thick white arrow.



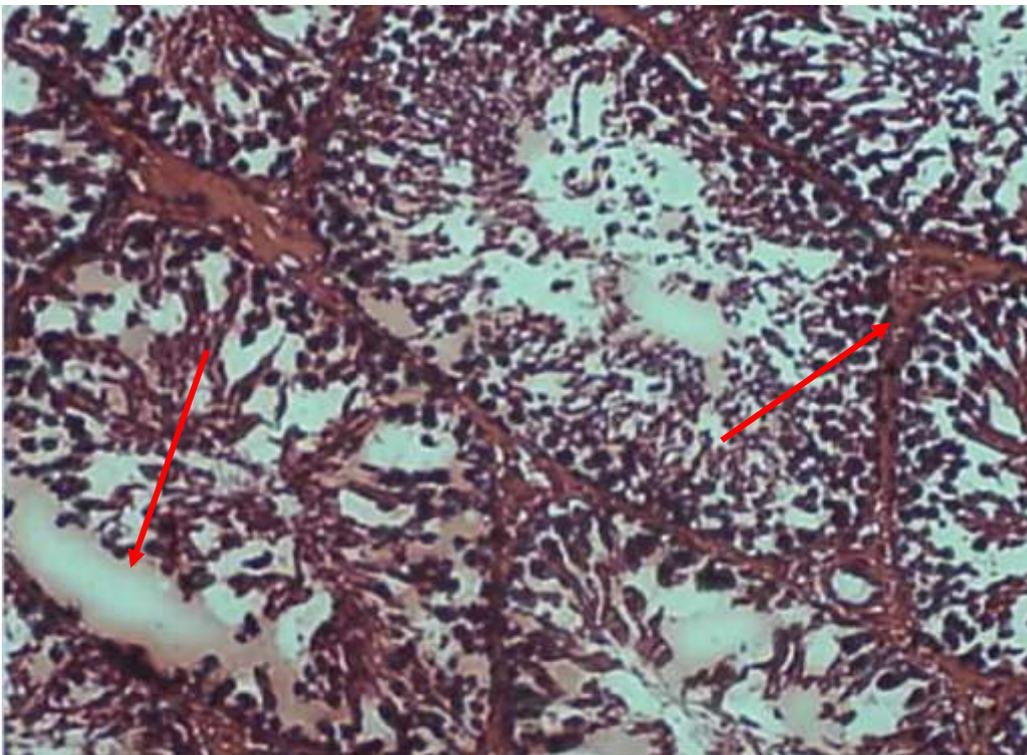
**Picture (3) testis section of male rat (group treated with acrylamide10% only) X40, show infiltration of inflammatory cells and degeneration of new sperm**



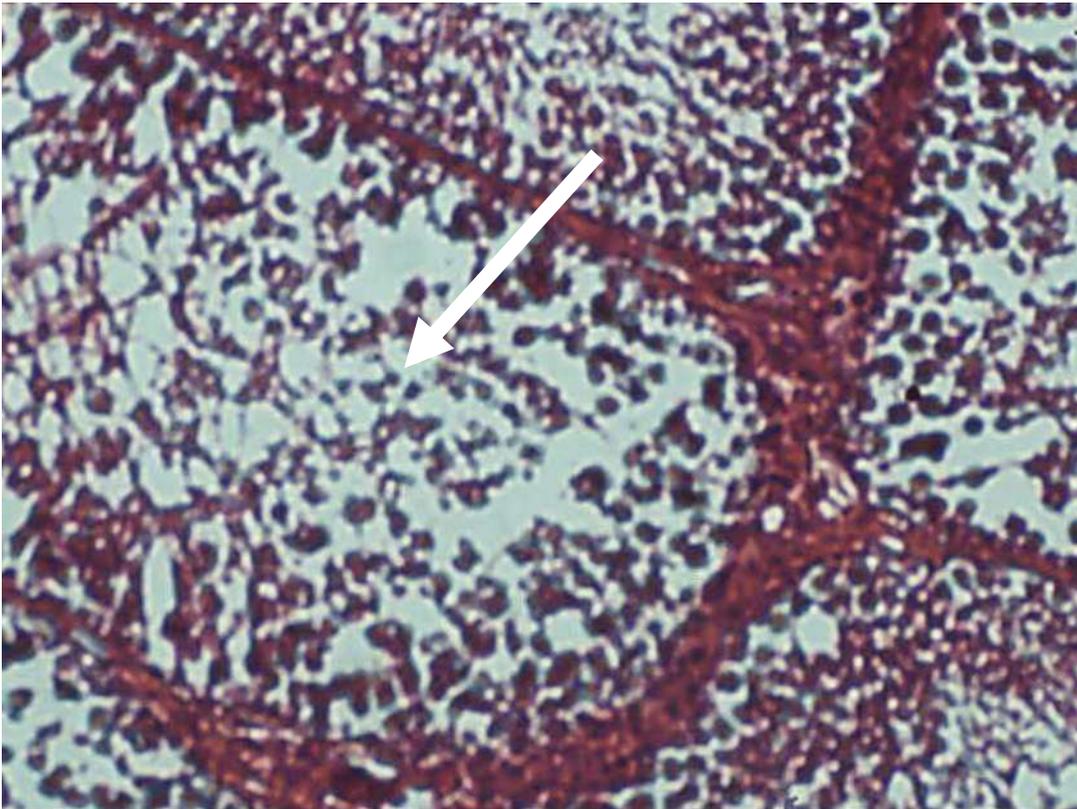
**Picture (4) testis section of male rat( group treated with acrylamide10% only) X40, show accumulation of edema and hemosedrine**



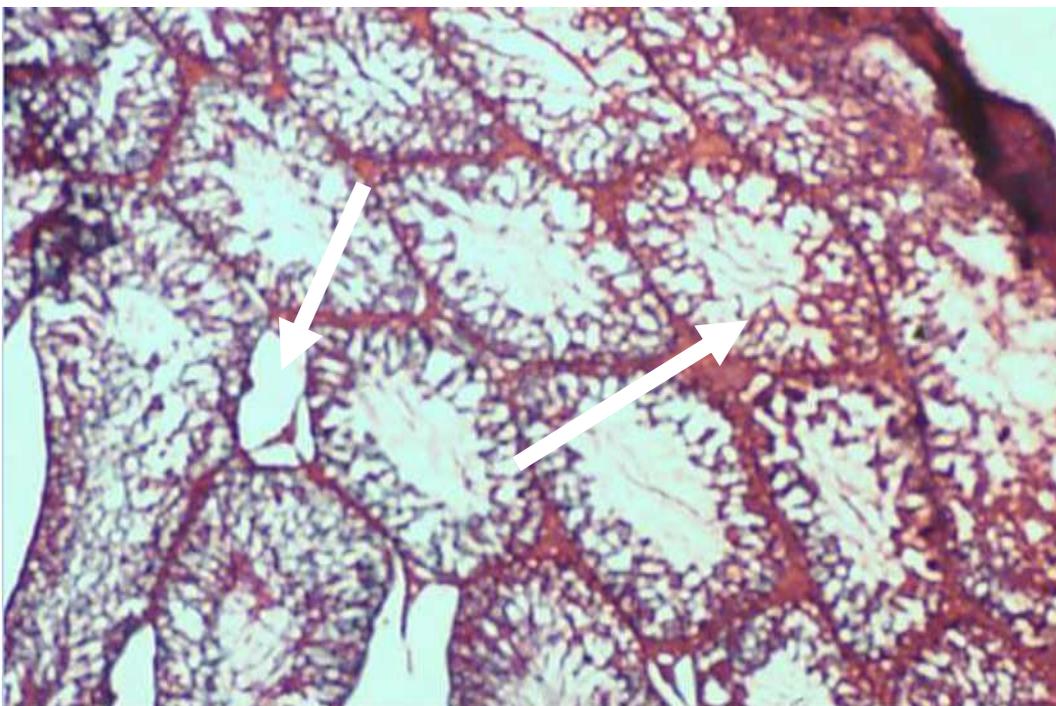
**Picture (5) testis section of male rat (group treated with acrylamide10% only) X40, show a wide necrosis area.**



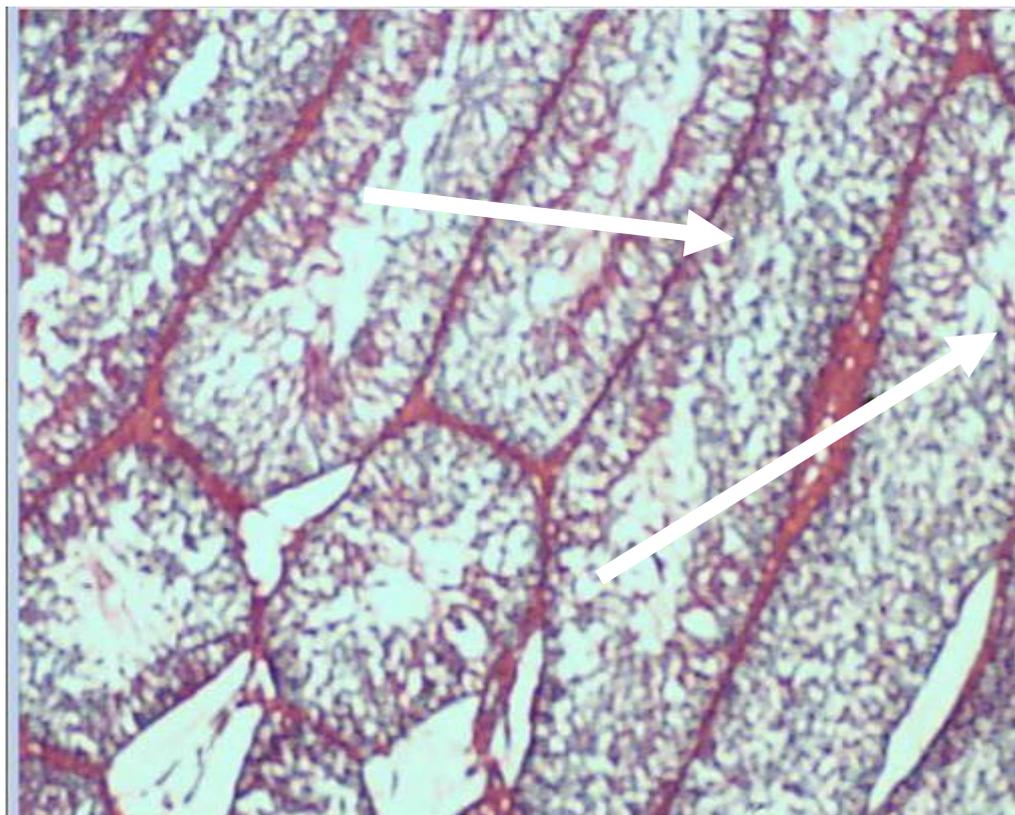
**Picture (6) testis section of male rat (group treated with acrylamide10% and omega 3) X40, demonstrated simple degeneration of spermatogenic cyst and mild hemosiderin**



**Picture (7) testis section of male rat (group treated with acrylamide10% and omega 3) X40, normal stroma and normal spermatogenesis.**



**Picture (8) testis section of male rat (group treated with omega 3 only) X10, there was normal stroma connective tissue and normal spermatogenesis**



**Picture (9) testis section of male rat (group treated with omega 3 only) X10, there was normal stroma connective tissue and normal spermatogenesis.**

### **Discussion:**

The above results refers to hazards effects of acrylamide on male rat reproductive system demonstrated from un recover pathological changes in slide section and significant decrease in testosterone hormone level as compared with control and other treated groups, so there are several theories may explain or give appropriate discussion to the above can be explain as the following: first of all agency for research on cancer classified acrylamide as a probable human carcinogen, on the other basis of its carcinogenicity in rodents(20,21), experimental animals studies showed that acrylamide could induce an increased incidence of cancer of the brain and the central nervous system, the thyroid and other endocrine glands, and reproductive organs of mice and rats(22). The mechanism by which acrylamide causes un recover pathological changes in laboratory animals and by which it may cause the same hazardous effect in humans is as yet un clear(23), but there are several pathways suggested such as acrylamide reacts with glutathione and gene transcription, or it may interfere with DNA repair or hormonal balance(24). However the reproductive toxicity of acrylamide is reported to induce dominant lethal mutations in spermatids (clastogenic and chromosome damaging effects) of mice and rats and is thus considered to be a mammalian germ cell mutagen (25,26), so acrylamide and glycidamide are reported to modify DNA both in vitro and in vivo (26). Binding of acrylamide (C14) to DNA of mice and rats were significantly greater after topical (dermanl) than after oral administration (27). Acrylamide itself react with DNA in vitro resulting in the formation of adenine and cytosine derivatives after hydrolysis (28). Acrylamide also induce morphological changes and reduction in glutathione GSH levels in Syrian hamster embryo cells. These changes were ameliorated by co-addition of N-acetyl-L-cysteine(27). Other possible modes action of acrylamide was investigated. These efforts were focused on the potential for acrylamide ACR to interfere with normal hormonal balance and the downstream consequences of that interference. Administration of ACR may prompt a biochemical cascade of events that, through feedback loops in hormonal control, results in an enhanced hormonal response. Several

experimental observations indicate that ACR may produce alterations in hormone levels that can impact several organs and organ systems. In particular, the decreases in serum prolactin and testosterone levels, in combination with changes in testes size and weight, suggest a connection between ACR's action at the neurotransmitter level and these effects (26, 29, and 30). This biochemical cascade would begin with the reduction in serum prolactin levels, which may be centrally mediated. In the study by Friedman *et al.* (31), dose-related decreases in testosterone to 53% of the control level occurred following a 14-day administration of ACRs which belong to the interference of acrylamide with neurotransmitter. It has been shown that an increased dopamine signal results in decreases in serum testosterone levels in male rats and it has been proposed that decreases in testosterone levels result in a down regulation of LH receptors on Leydig cells (30,31). According to Cook *et al.* (29), decreases in testosterone production and result in a compensatory increase in serum LH to maintain testosterone at physiological levels. The sustained compensatory increase in LH results in increases in Leydig-cell hyperplasia (32). In the other hand there are no un recover pathological changes in histological testis section for group drenched with omega 3 only as well as group drenched with omega 3 and acrylamide 10% as compared with the other group, however there are limited researches and studies which are explaining the promotion effect of omega 3 fatty acids on testosterone levels can be included as follow: nutrition have a vital role on endocrine glands functions, so the evolve levels of testosterone by the effect of polyunsaturated fatty acids on increase content of phospholipids and decrease accumulation of peroxidized and cross linked fatty acids, trans – fatty acids, and cholesterol esters in biological membranes(33,34,35). These alteration lead to increase of membrane fluidity (36 and 37). Cell membrane fluidity can be improved by increasing dietary PUFA (32), and changes in the fatty acid content of cell membranes can be achieved fairly rapidly (4-8 weeks) by altering dietary fat intake(35,36). In old rats, substitution of corn oil, which is high in omega 3(PUFA), for 12 weeks, resulted in near-normalization of the glycogenolytic response of liver cells to glucagon and of the lipolytic response of fat cells to beta-adrenergic stimulation (38 and 39), while the other reason may be acceptable to give a reliable explanation that omega 3 fatty acids have a powerful initiation effect on acetylcholine which is a neurotransmitter regulation sexual desire, so stimulation this neurotransmitter has a positive effect on cognitive functioning, especially memory and attention, and also increase semen volume, in other hand omega 3 fatty acids have a promotion effect on serotonin which is another neurotransmitter synthesized from the amino acids tryptophan in central nervous system, as well as gastrointestinal tract, this neurotransmitter which effect on increase sex hormones, so it is recommended not to take its orally because serotonin not has ability to cross blood brain barrier directly when taken orally, however tryptophan and its metabolite 5-hydroxytryptophan (5-HTP), from which serotonin is synthesis, can and do cross the blood brain barrier. These agents are available in dietary supplements like omega 3 (40,41and42).

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