

## **Effect of acrylamide and Pomegranate Juice on Reproductive Efficiency of Adult Rats**

### **دراسة تأثير اعطاء الاكراليميد والاكرايمايد مع عصير الرمان في الكفاءة التكاثرية لذكور الجرذ**

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#### **Abstract**

The study was conduct to investigate the possible protective effect of pomegranate juice after experimental oral administration of the acrylamide on the serum reproductive hormones levels (Testosterone "T", Luteinizing Hormone "LH" and follicle- stimulating Hormone" FSH"), and epididymal sperm concentration, viability and abnormalities as well as fertility in adult male rats. Forty-eight adult male albino rats were divided equally into four groups (12 male rats /group): negative control group (G1), which was given distilled water (3 ml/kg/ body weight "BW"), and the positive control group (G2) given oral pomegranate juice (3 ml/kg/ BW). The others were considered as treated groups, third group (G3) was administered acrylamide (100 mg/kg/day), and fourth group (G4) given acrylamide in the same dose combined with (3 ml/kg/BW) of pomegranate juice daily both for 30 days. All administrations were given by oral gavage. Six male rats from each group were sacrificed perform hormonal tests and sperm count and other 24 males (6 male of each group) were mated with 24 untreated healthy adult female rats in order to evaluate fertility efficacy of males in studied groups. The results showed significant decrease ( $P \leq 0.05$ ) in serum T levels, and significant increase ( $P \leq 0.05$ ) in LH and FSH levels in G3 group compare to all other groups of study. The results also showed significant decline in epididymal sperm concentration and viability concurred with significant elevation in sperm abnormality in (G3) group compared to other treated and control groups. Results also revealed that the treatment with pomegranate juice improved the harmful effects of acrylamide on reproductive parameters in male rats toward the normal values. Fertility rate, number of birth and implantation sites were reduced in female rats that were mated with male of G3 group in comparison with females that were mated with males of G1, G2 and G4 groups.

From the present study, it has been revealed that the acrylamide adversely affect male reproductive efficacy and caused decline their sperm characters and subsequent fertility. Moreover, Pomegranate Juice had the ability to prevent these changes. Thus, it could have a role in improving male fertility.

#### **الخلاصة**

اجريت الدراسة الحالية لدراسة الدور الوقائي المحتمل لعصير الرمان ضد التأثيرات الضارة المستحثة بواسطة مادة الاكراليميد على هرمونات التكاثر ( الشحمون الخصوي ' هرمون الاباضة والهرمون المحفز للجريب) و على تركيز النطف وحيويتها وتشوهاتها اضافة الى الكفاءة التكاثرية في ذكور الجرذان. استخدمت في هذه الدراسة ثمان واربعون ذكرا من الجرذان البيض قسمت عشوائيا الى اربع مجاميع متساوية (12 جرذ لكل مجموعة) وعلى النحو التالي : مجموعة السيطرة السالبة : أعطيت ماء مقطر فقط , ومجموعة السيطرة الموجبة : اعطيت عصير الرمان فقط بجرعة (3 مل/كغم من وزن الجسم) المجموعة الثالثة : اعطيت الاكراليميد فقط (100 ملغم / كغم من وزن الجسم ) المجموعة الرابعة : أعطيت الاكراليميد بنفس الجرعة المستعملة في المجموعة الثالثة ثم أعطيت عصير الرمان بنفس الجرعة المستعملة في مجموعة السيطرة الموجبة من وزن الجسم يوميا بواسطة التجريع عن طريق الفم لمدة 30 يوما. قتلت 6 ذكور من كل مجموعة عند نهاية لاجراء فحوصات الهرمونات وتحليل النطف البربخية, وتركزت الذكور الاربعة والعشرون المتبقية الاخرى ( 6 ذكر من كل مجموعة)

لغرض التزاوج مع 24 انثى سليمة وغير مجرعة بهدف تقييم خصوبة الذكور. أظهرت نتائج الدراسة ان اعطاء الاكرليمايد لوحده قد تسبب في انخفاض معنوي في مستوى هرمونات الشحمون الخصوي وارتفاعا معنوياً في مستويات هرموني اللوتيني والمحفز للجريب كذلك سبب تدهوراً في صفات النطف اذ انخفض كل من تركيز النطف وحيوية النطف معنوياً مصحوباً بارتفاع النسبة المئوية للنطف المشوهه مقارنة مع مجموعة السيطرة السالبة والموجبة في حين لوحظ ان اعطاء عصير الرمان مع الاكرليمايد قد ادى الى تحسين التأثيرات الضارة التي سببها الاكرليمايد على بعض معايير الجهاز التناسلي الذكري للجرذان باتجاه القيم الطبيعية. كما بينت نتائج الدراسة ايضاً وجود انخفاض في معدلات الخصوبة وعدد المواليد وعدد مواقع الغرس في الاناث التي زوجت من ذكور معالجة بالاكرليمايد مقارنة بالاناث التي زوجت من ذكور مجموعتي السيطرة الموجبة والسالبة كما بينت الدراسة ان خصوبة الذكور عادت الى المستوى الطبيعي في المجموعة الرابعة. وقد خلصت الدراسة الحالية الى ان لمادة الاكرليمايد تأثير ضار على الكفاءة التناسلية لذكور الجرذ الأول وان عصير الرمان خفض كثيراً من هذه التأثيرات.

## **Introduction**

The increasing number of occurrences of impairment in the reproductive functions in humans and wildlife observed over the past few decades has raised concerns about certain chemicals in our environment (1). Acrylamide is a vinyl monomer, which exists, in the form of a white crystalline powder (2). Previously, acrylamide exposure was thought to occur mainly through occupational exposure. However, Acrylamide can be detected in starchy foods treated at high temperatures (120°C). Exposure to acrylamide may accrue in factories, laboratories, or daily life via food and drinking water (3). Acrylamide is neurotoxic to experimental animals and humans (4) and has mutagenic and carcinogenic effects (5). Recently, attention has been drawn towards the toxicity of acrylamide (6, 7).

The neurotoxicity of acrylamide in adult animals has been well investigated. On the other hand, information about acrylamide-induced effects on male reproductive indices is relatively limited.

Pomegranate (*Punica granatum*) is widely used in the folk medicine of many cultures especially in the Middle East. Pomegranate has high content of antioxidant that includes tannins, flavonoids and anthocynins (8). The content of soluble polyphenols in pomegranate juice varies within the limits of 0.2% to 0.1% including mainly tannins, ellagic tannins, anthocyanins, catechins, gallic and ellagic acids (9). Pomegranate exhibits antiviral, antioxidant, antidiabetic, antidiarrheal, anti-cancer and antiproliferative activities (10, 11, 12).

The nutritive value of the pomegranate fruit was demonstrated and that a glass of pomegranate juice provides approximately half of an adult's recommended daily allowance (RDA) of the vitamins A, C and E (13) and the antioxidant polyphenols responsible for about half antioxidant ability of the fruit to scavenge free radicals (14). In addition, many factors such as storage of the fruits and industrial food processing have ability to affect the content of these antioxidants (15). The link between the antioxidant-rich juice and male fertility have an important role to solve male fertility problems in recent research work (16), which could make pomegranate an important food supplement. The current study aims to evaluate the harmful effect of acrylamide on male fertility as well as for evaluating the possible protective effect of pomegranate juice against acrylamide-induced reproductive toxicity in male albino rats.

## **Materials and Methods**

The study included 48 mature male and 24 mature female *Rattus norvegicus* rats were, provided by College of Medicine / University of Baghdad, They were 16 to 18 weeks old. The animals were clinically healthy, kept under hygienic conditions, housed in metal cages in animal house of the College Veterinary Medicine University of Kerbala. The animals were accommodated to the laboratory conditions for 14 days before beginning of experiment. The light system was 12/12 hrs light/dark, with food and water freely available.

Acrylamide were dissolved in distilled water to achieve the desired concentrations. Fresh solution were prepared weekly and stored in glass containers. Based on the body weight of male rats dose was administered to each one. Pomegranate Juice was prepared according (17). The duration of the oral administration during the experiments lasts for 30 day.

The present study consists of two experiments as following:

Experiment one in which 48 male rats, were randomly divided into four groups: first group (G1) (negative control group): included 12 rats that received a daily oral gavage of distilled water (3ml/kg/BW) vehicle. Second Group (G2) (positive control group): included 12 adult male rats that received a daily oral gavage of pomegranate juice in a dose of (3ml/kg/BW) according to (17). Third group (G3): included 12 rats it received acrylamide at a dose of 100 mg /kg /BW. Fourth group (G4): it included 12 rats were given an oral daily dose of (3ml/kg/BW) pomegranate juice combined with acrylamide in the same dose of G3.

At the end of experiment, blood was collected via heart puncture from six anesthetized male rats of each group to perform hormonal investigation. Then after, these rats were sacrificed and testis trimmed out to use for sperm characters. Other six males of each group were used to perform experiment two (fertility).

Experiment two (fertility study) in which six males of each of above groups and twenty- four untreated female rats were used (one male with one untreated female in a separate cage for 14 days). Vaginal smears were done to detect pregnant female. Sperm-positive females were housed separately and killed after delivery. The number implantation sites and resorption sites was detected according to 18. The following parameters were measured: Number of delivering females, Fertility percent, Litter size, Number of total implantation site, Number of resorption sites

Estimation of serum testosterone, luteinizing hormone (LH) and Follicles-stimulating hormone (FSH) levels by follow manufacture instructions of kit by method mentioned by Teitz (19). All kits used for hormone assay were (Monobind Inc. lake forest CA 92630, USA).

Seminal content of epididymis was obtained by cutting of cuda epididymis using surgical blades and squeezed in a sterile clean watch glass. This content was diluted 10 times with 2.9 % sodium citrate dehydrate solution and thoroughly mixed to estimate sperm concentration (20). One drop of the suspension was smeared on a glass slide and stained by Eosin-nigrosin stain to determine the percentage of sperm cell viability and morphological abnormalities (21). Abnormalities of sperms were evaluated according to (22)

The results were expressed as mean  $\pm$  stander error (SE). The comparisons between groups were performed with analysis of variance (ANOVA) by using computerized SPSS program (Statistical Program for Social Sciences).  $P < 0.05$  was considered to be lest limit of significance. Least significant different test (LSD) was calculated to test difference between means (groups) for (ANOVA) (23)

## **Results and Discussion**

Testosterone is a steroid hormone regulates sperm production within the testes. It is produced by interstitial cells and its secretion regulated by hormones of pituitary gland (24).

Table (1) showed that serum testosterone level significantly decreased ( $P \leq 0.05$ ) in male rats exposed to acrylamide. These changes matched with results of (25) who concluded that acrylamide caused a testicular damage in mice and with results of (26), (27) and (28) who illustrated the relationship between acrylamide and reproductive toxicity in rats. Moreover, Song *et al.* (29) in exploring the effect of subchronic exposure to acrylamide on the reproduction and testes endocrine function in rats, he reported a marked reduction in testosterone level in the serum as well as in the testes homogenates. The significant reduction of testosterone may be a result of direct damage of acrylamide on the Leydig cells( 26). In addition, acrylamide may affect the endocrine function of the testes. It may interfere with the biosynthesis of androgen, which mediated by cytochrome P-450 system of interstitial cells of the testes which is required for the functioning of 17  $\alpha$  hydrolase enzyme and 17-20 hydrolyase enzyme (30), or induces a variety of hepatic biotransforming enzymes, which are capable of metabolically transforming androgens into products with low androgen receptor binding activity (31). FSH and LH secretion affected by a negative feedback from sex steroid hormones (26). Therefore the significant increase of serum level of these hormones in acrylamide group (table 1) may be due insufficient level of testosterone which to initiate negative feedback mechanism. On the other hand, an increase in stress hormones, such as cortisol, leading to

a subsequent decrease in another hormone called gonadotropin releasing hormone (GnRH). GnRH is made in the hypothalamus and it plays a role in the production of key hormones (LH and FSH) that can affect the quality and quantity of sperm. This stress/fertility link has been fairly well established in years past (32). Stress reduces hypothalamic-pituitary-gonadal axis activity due to the antagonistic relationship between testosterone and corticosteroids (33). Co-administration of pomegranate juice to acrylamide treated group rats restored the sperm characteristics towards normal values. This increase of testosterone level in the present study due to pomegranate juice can be in part due to the ability of pomegranate to reduce stress caused by acrylamide administration.

Table (1). The Effect of acrylamide and pomegranate juice on Reproductive Hormones Levels in male Rats (Means  $\pm$  SE).

Parameter Groups	Testosterone ng/ml	LH $\mu$ IU/ml	FSH $\mu$ IU/ml
G 1 ( Negative Control)	A 3.49 $\pm$ 0.37	B 1.29 $\pm$ 0.11	B 1.54 $\pm$ 0.14
G 2 (Positive Control)	A 3.75 $\pm$ 0.53	B 1.19 $\pm$ 0.08	B 1.35 $\pm$ 0.12
G 3 (Acrylamide only)	B 1.23 $\pm$ 0.48	A 1.78 $\pm$ 0.08	A 2.37 $\pm$ 0.10
Group 4 (Acrylamide +Pomegranate juice)	A 3.11 $\pm$ 1.19	B 1.31 $\pm$ 0.05	B 1.51 $\pm$ 0.09
LSD	1.22	0.38	0.69

N=6

Different letters in the same column represent a significant difference at ( $p \leq 0.05$ )

Spermatozoa are especially susceptible to peroxidative damage because of the high concentration of polyunsaturated fatty acids, which are involved in regulation of sperm maturation, spermatogenesis, capacitation, acrosome reaction and eventually in membrane fusion and low antioxidant capacity. Obviously, peroxidation of sperm lipids destroys the structure of the lipid matrix in the membranes of spermatozoa, it is associated with the rapid loss of intracellular ATP leading to axonemal damage, decreased sperm viability and increased mid-piece morphological defects, and even it completely inhibits spermatogenesis in extreme cases (34). Results showed that oral administration of acrylamide (100 mg/kg B.W) caused highly significant reduction in the sperm parameters compared with control group rats (table 2). This result matched with other previous studies, where acrylamide use was associated with decreased sperm parameters (35,36,37). The reduction in the sperm parameters in the present study may occur as a result of decrease in the serum testosterone levels which induced by acrylamide uptake which leading to degeneration of seminiferous tubules with sloughing of seminiferous epithelium and spermatogenic cells (38). A relevant level of testosterone in testes is essential for normal spermatogenesis, maintenance of sperm morphology and normal physiology of seminiferous tubules (39) administration of pomegranate juice (positive control) enhanced the sperm characters compared with negative control group and Co-administration of pomegranate juice to acrylamide treated group rats restored the sperm characteristics towards normal values( table 2). All these activities may be related to diverse phenolic compounds present in pomegranate juice, including punicalagin isomers, ellagic acid derivatives and anthocyanins (delphinidin, cyaniding and pelargonidin 3-glucosides and 3,5-diglucosides). These compounds are known for their properties in scavenging free radicals and inhibiting lipid oxidation in vitro (40). Vitamin A, provided by pomegranate juice, is required for the maturation process of epididymal spermatozoa, together with the potent antioxidant role of pomegranate juice (41) might explain the protective effect of this juice on sperms. Such improvements in the semen characteristics by Pomegranate juice agree with previously reported benefits of pomegranate juice on sperm quality and male fertility of rats (16, 42).

Table (2): The Effect of acrylamide and pomegranate juice on sperm characters in Rats (Means  $\pm$  SE)

Parameters Groups	Sperm concentration $\times 10^6/\text{mm}^3$	Viability %	Abnormality %
G 1 Negative Control	B 2.09 $\pm$ 0.41	B 70.61 $\pm$ 2.49	B 11.05 $\pm$ 2.08
G 2 (Positive Control)	A 2.93 $\pm$ 0.19	A 81.76 $\pm$ 2.52	B 10.71 $\pm$ 3.55
G 3 (Acrylamide only)	C 1.15 $\pm$ 0.29	C 45.53 $\pm$ 2.18	A 33.53 $\pm$ 2.94
Group 4 (Acrylamide +Pomegranate juice)	B 2.11 $\pm$ 0.25	B 69.15 $\pm$ 2.75	B 15.29 $\pm$ 3.02
LSD	0.73	10.65	9.69

N=6

Different letters in the same column represent a significant difference at ( $p \leq 0.05$ )

In concern with male fertility, result of the present study revealed that the females of control group mated with males that had exposed to acrylamide were showed significant reductions in pregnancy rates, litter size and total number of implantations (table 3). The reason for treated rat's subfertility in the present study could be due to impaired spermatogenesis caused by damage of leydig cells as a result of exposure to acrylamide, and / or due to reduction in serum testosterone level in male rat as well as low sperm count which supports by hormonal and sperm characters (table 1 , 2). It has been found that the principal cause of idiopathic male infertility is an underlying pathological condition known as "oxidative stress". Acrylamide have been shown to induce oxidative stress, which was postulated to be associated with male infertility (43). In conclusion, the present study showed that Acrylamide produced remarkable changes in the hormone of reproduction and causing marked decrease in the number of caudal sperm concentration and viability as well as increase in sperm abnormality. It is assumed that these changes would interfere with the proper function of the testis and accordingly contribute to infertility. On the other hand, the results obtained proved the pomegranate juice potentials to counteract all acrylamide induced changes thus allowing recovery of the rat from oxidative stress and hence contributing in improving male fertility. Therefore, it is recommended to supplement pomegranate juice in male individuals suffering from infertility.

Table (3) The Effect of acrylamide and pomegranate juice on fertility outcome of non- treated female mated with male of different groups in study (Means  $\pm$  SE)

Parameters Groups	No. of no treated females	No. of delivered female	Fertility rate %	Total No. of Implantation sites (mean $\pm$ SE)	No. of birth(mean $\pm$ SE)	No. of Resorption Site (mean $\pm$ SE)
Female mated with male of G1 group	6	5	83.3%	A 9.16 $\pm$ 1.53	A 7.66 $\pm$ 1.22	A 1.5 $\pm$ 0.34
Female mated with male of G 2 group	6	6	100%	A 10.33 $\pm$ 0.71	A 8.66 $\pm$ 0.66	A 1.67 $\pm$ 0.33
Female mated with male of G 3	6	2	33.3%	C 2.5 $\pm$ 1.62	C 1.33 $\pm$ 0.98	A 1.17 $\pm$ 0.74
Female mated with male of G4 group	6	4	66.6%	B 7.33 $\pm$ 2.10	B 5.5 $\pm$ 1.52	A 1.83 $\pm$ 0.60

Different letters in the same column represent a significant difference at ( $p \leq 0.05$ ).

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