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Prophylactic Role of Resveratrol against Testicular toxicity induced by Acrylonitrile in Rats

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Abstract

Acrylonitrile (ACN) is an aliphatic nitrile product which is extensively used in various synthetic chemical industries. ACN is known to exert toxic actions to human beings and experimental animals. The present study was designed to examine the ability of Resveratrol to protect testicular tissue from the toxic effects of ACN in adult albino rats. Groups of rats were submitted to daily oral administration of ACN for 90 days at a dose level of 40 mg/kg b.w., while other group submitted to daily co-administration of resveratrol in a dose 20 mg/kg b.w. with ACN 40 mg/kg b.w.. The results showed significant reduce in the levels of serum Testosterone (T), follicle-stimulating hormone (FSH) and luteinizing hormone (LH) in ACN group which indicates injury of the testicular tissue characterized by atrophy of seminiferous tubules, depletion and degeneration of germ cells and spermatocytes, while the interstitial tissue showed edema, congestion of blood vessel and cluster of Leydig cells with irregular pyknotic nuclei and separation of seminiferous tubules germinal layers from the basement membrane. Resveratrol reduce the effects of ACN and enhanced sex hormone production T, FSH and LH and enhanced the histopathological changes characterized by atrophied seminiferous tubules, mild absence of spermatid and sperm stages, edema and congestion of blood vessel of interstitial tissue.In conclusion, ACN cause severe testicular damage indicated by sex hormone production and histopathology, while resveratrol improve hormone production and reduce pathological effects of resveratrol.

Keywords: Acrylonitrile, Resveratrol and Testis.

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Introduction

Acrylonitrile (ACN) is highly poisonous compound with formula (CH3H3N), having active vinyl and cyanide groups, extensively used in the manufacturing of acrylic fibers, resins and plastics, and has been found in drinking water, food products, cigarette smoke and air pollutants[1,2]. physical properties of acrylonitrile homopolymer which is white or yellow opaque material with strength, general insolubility and high softening temperatures[3], due to the presence of polar cyano groups (C N) in acrylonitrile units which increased inter chain forces leading to increase softening points[4] . Human exposure to ACN could potentially occur during the manufacturing process, end product usage and transportation. Also near chemical waste sites which it has been improperly stored or disposed, two most likely exposure pathways are breathing acrylonitrile that has evaporated in to air or drinking water that has been contaminated due to ACN is highly soluble and stable in water. Further, such exposure can also be possible in the general population through cigarette smoke and *via* contamination of drinking water[5] Studies have shown that occupational exposure to ACN can lead to neurotoxicity[6], immunotoxicity [7], and gastric toxicity[8]. Epidemiological studies reported that there are adverse reproductive effects, such as infertility, sex hormone decline and birth defects, after exposure to ACN[9] previous studies demonstrated that ACN has the potential to induce testicular toxicity[10]. The metabolism of ACN is mainly carried out in two different ways, i.e. the conjugation with glutathione (GSH) directly, and the epoxidation catalyzed by cytochrome P-450 (CYP-450) to cyanoethylene oxide (CEO) [11]. The metabolism consumes GSH with a release of cyanide (CN−), which causes a generation of reactive oxygen species and initiates a free radical reaction cascade, making the body undergo a lipid peroxidation[12]. The depletion of GSH and free radicals generated from metabolism of ACN leads to oxidative damage[13]. Resveratrol (trans-3,5,4′-trihydroxystilbene; RES) is polyphenolic phytoalexin and found largely in grapes. A product of grapes, red wine also contains significant amount of resveratrol[14]. It has been classified as a stilbeniod, a type of natural phenolic compound a well-known natural compound that has been used to alleviate numerous disease conditions, also widely used in medical fields as an antioxidant[15, 16]. The beneficial effects of RES have been referred to its capability to pressures that are considered most likely in mammalian cells. Therefore, the aim of the current study was to investigate the efficacy of RES on ACN-induced functional and structural alterations related to oxidative stress in the testes of rats. Resveratrol act as a chemo preventive [17],anti-inflammatory[18],anti-hyperlipidemia, cardio protective, immunomodulatory, anti-diabetic[19], anti-

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oxidant agent[20] and anticancer activity a potent anti-oxidant agent and resveratrol with doxorubicin chemotherapeutic drug in-vitro had enhanced the cytotoxic activity of such a chemotherapeutic drug, while simultaneously eliminating its cardiotoxicity side effect[21]. In healthy rodents, RES has a good impact on most semen parameters, sperm production, testosterone levels, and penile erection in control or stress conditions[22, 23]. In an experiment performed on animal models of cadmium chloride-induced reproductive toxicity, RES protected rat's testis and restored steroidogenesis and reproductive function via an anti-oxidant potential and inhibition of apoptosis[23].

Materials and methods

Chemicals

Acrylonitrile (ACN) and was obtained from (Sigma–Aldrich, Germany) and given by oral gavage at dose levels of 40 mg/kg b.w. for 90 day[24]. It was prepared by dissolving of 0.8 ml of AN in 100 ml of D.W. (0.8% v/v). Resveratrol was obtained from (Now company, USA) administered orally by gavage 20 mg/kg body weight daily for 90 days[25]. The doses were freshly prepared immediately before administration.

Experimental animals

Fifty-five male Sprague-Dawley rats, each weighing 260±10 g, were obtained from the Breeding Unit of the Higher Institute for the diagnosis of infertility and assisted reproduction techniques, Al- Nahrain University. The animals were housed in the animal house of college of Veterinary Medicine-University of Baghdad in standard cages after grouping in batches of five under special conditions of relative humidity (50±5%), temperature (25±3 °C) and a 12 h light/12 h dark cycle. Rats were allowed free access to standard commercial pellets and tap water and were acclimatized to laboratory conditions for a period of one week before the onset of experimentation.

Experimental protocol

The rats were divided into four groups as following:

Group I (Control): (n=10) rats were served as negative control.

Group II (Resveratrol group): (n=15) rats were administrated single dose of Resveratrol (20mg/kg) through oral route for 90 days.

Group III (ACN group): (n=15) rats were administrated single daily dose of ACN (40mg/kg) through oral route for 90 days.

Group IV (ACN +Resveratrol group): (n=15) rats were administrated orally single dose of ACN (40mg/kg) and Resveratrol (20 mg/kg) for 90 days.

At day 90 post administration blood collected from all groups for the detection of sex hormone (T, FSH and LH). Then rats were euthanized by intraperitoneal injection of ketamin 90 mg/kg

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B.W. and Xylozine 40 mg/kg B.W. to obtain testes form the rats.

Hormonal assay

Determination of testosterone (T), follicle stimulating hormone (FSH) and luteinizing hormone (LH) (MybioSource, Netherlands) in serum were carried out using ELISA technique according to [26, 27]

Ethics approval:

This experiment was done in accordance with ethics approval committee of the animal care at College of Veterinary Medicine University of Baghdad.

Microscopical examination of the testes

For testicular histology examination, samples of tissue from the testes were immediately fixed in 10% neutral buffered formalin for 24 hr and routinely processed, sectioned (4-6) µm and stained with haematoxylin and eosin[28]. The slides were examined by light microscop (Olympus, Japan) and photographed by 17-megapixel microscopic camera (Omax, china).

Statistical analysis

The data was tabulated in a datasheet of IBM SPSS version 26.0, which was utilized to do the statistical analysis. The mean and standard errors of continuous variables were reported, and significant differences were tested using the analysis of variance (ANOVA) test, followed by the least significant difference (LSD) test. Statistical significance was defined as a probability value ($p \leq 0.05$).

Results

Hormonal assay

Data listed in Table 1 show that treatment with ACN caused a significant ($P < 0.001$) decrease in the levels of T, FSH and LH, as compared to the corresponding control group.

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Hormone / Groups	Testosterone (ng/ml) Mean ±SE.	L.H (mIU/ml) Mean ±SE.	FSH (mIU/ml) Mean ±SE.
G1	7.95±0.09	8.56±0.76	9.55±0.07
G2	9.76±0. 07	9.98±0.08	11.77±0.06
G3	3.53±0. 08	3.51±0.08	3.63±0.07
G4	4.83±0. 02	6.73±0.09	6.32±0.09
p--value	0.000*	0.000*	0.000*

Pre- and co-administration of RES to ACN-challenged rats significantly improved Microscopical studies of the testes

Microscopicaly, resveratrol group showed normal morphology of the seminiferous tubules including all stages of the spermatogenic cycle (Figs.1 a& b). However, animals treated with ACN showed atrophid seminiferous tubules, depletion and degeneration of germ cells and spermatocyte, while (Figs.2 C) the interstitial tissue showed edema, congestion of blood vessel and cluster of Leydig cells with irregular pyknotic nuclei, also congestion of blood vessel, edema and atrophy of Leydig cells of interstitial tissue, the seminiferous tubules contain degenerated spermatids and edematous fluid under tunica albuginea (Fig. 2 b), the seminiferous tubules showed fluid in the lumens (Fig. 2 e), separation of germinal layer in the basement membrane (Fig. 2 f). Rats treated with ACN/RES showed marked atrophid seminiferous (AST) tubules, mild absence of spermatid and sperm stages (Fig. 3 a), severe edema and congestion of blood vessel of interstitial tissue atrophy and necrosis of seminiferous tubules (Fig. 3 b).

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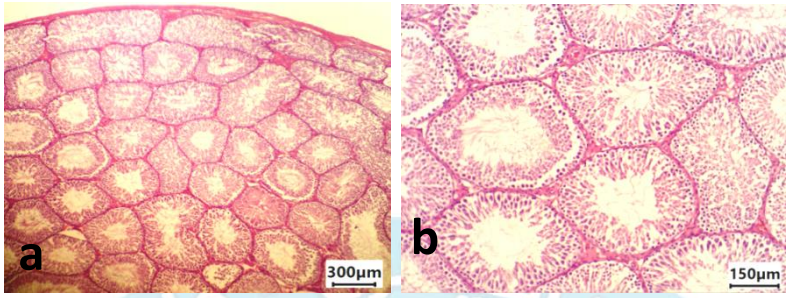

Figure 1: Microscopic section of control rat testes showed (a): regularly arranged seminiferous tubules (ST) and interstitial elements (IE) in between; (b): different stages of germ cell development: the Spermatogonia (Sg), spermatocytes (Sp) and spermatids (Sd), the supporting Sertoli cells (SC) are resting on the basal lamina.

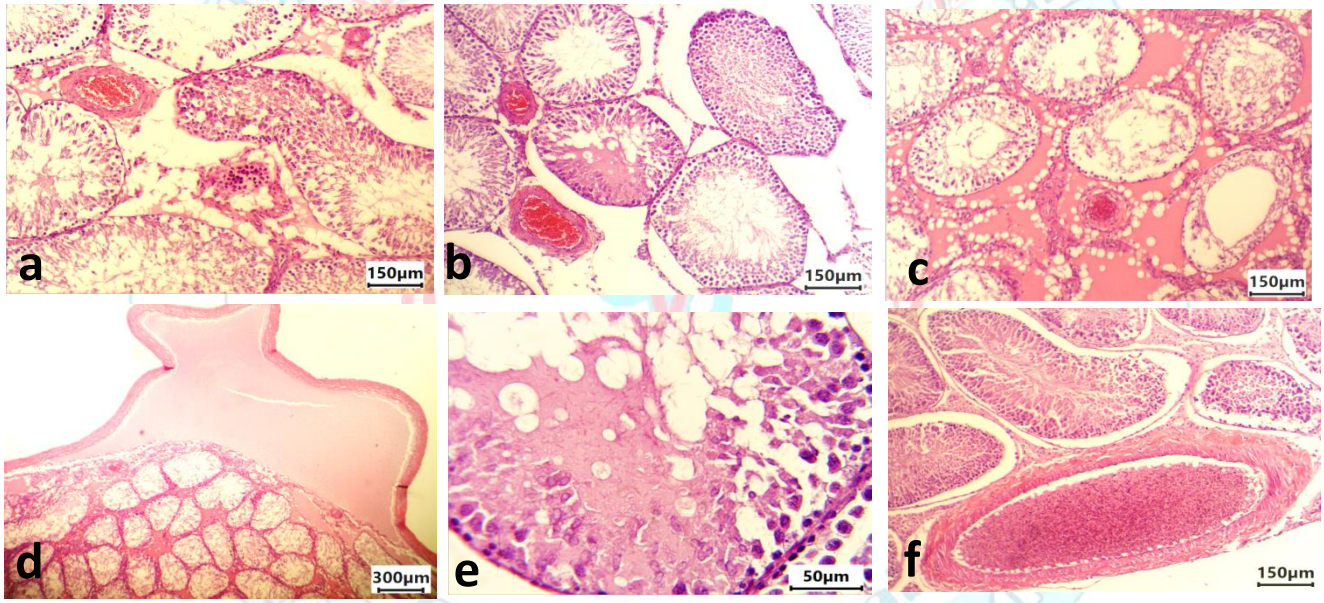

Figure 2: Microscopic section of rat testes from ACN-treated group showed: (a and b) severely damaged seminiferous tubules (asterisks) where they exhibit germ cell depletion, tubular atrophy and maturation arrest; (C) necrosis of almost all the spermatogenic cells is also visible in some tubules (arrows), congestion of the blood vessel and edema of the interstitial tissue; (d) edema under tunica albuginea; (e) edematous fluid in the seminiferous tubules; (f) separation of the germinal layer from the basement membrane.

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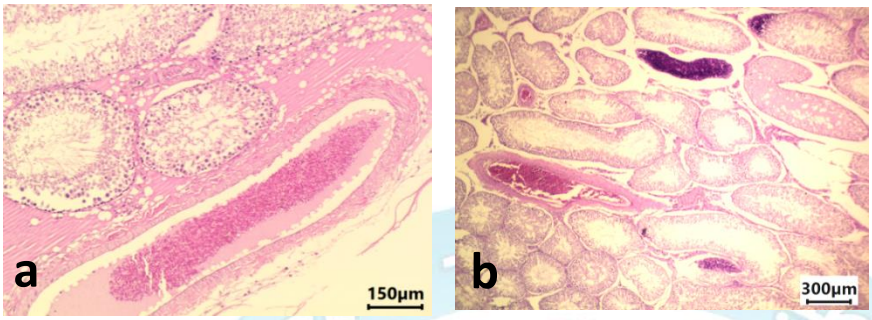

Figure 3: Microscopic section of rat testes from ACN-Resveratrol treated group showed: (a) atrophid seminiferous (AST) tubules, mild absence of spermatid and sperm stages; (b) mild edema and congestion of blood vessel of interstitial tissue (black arrow). (H and E)

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Discussion

It has been shown that acrylonitrile causes male reproductive damage in lab animals[29, 30]. and also in ACN-exposed workers [31]. [32] reported that altered spermatogenesis may occur in both humans and animals, and that endocrine maintenance is necessary for normal spermatogenesis.

Serum levels of T, follicle-stimulating hormone, and luteinizing hormone all dropped, as shown by the present research. The present results are in accordance with the study of [33] who reported that ACN decreases testosterone synthesis and/or secretion in humans. According to this research, the rise in oxidative stress may be responsible for the detected decrease in T levels after ACN administration. This result is in agreement with that of [34]who demonstrated that H2O2 is a powerful oxidant with the ability to reduce steroidogenesis (decrease the production of testosterone) in Leydig cells. Similar to this, concluded that rats administered with the structurally related vinyl monomer acrylamide (ACA) had lower blood T levels and Leydig cell. According to their research, ACA treatment reduced the viability of Leydig cells by decreasing testosterone levels, which in turn reduced spermatogenesis in the rat testes. [35]studies also showed a significant reduction in the blood levels of T, FSH, LH, and prolactin (PRL) in rats after ACA treatment. A decrease in T, FSH, LH, and PRL levels was ascribed by the author to pituitary gland malfunction, and it was also shown that ACA has an influence on the testes directly or indirectly by affecting the pituitary gland and lowering FSH and LH production. The current findings are also consistent with research by [36] that discovered cadmium decreased T production by lowering levels of cyclic adenosine monophosphate (cAMP) and LH receptor messenger ribonucleic acid (mRNA) in rats. The present research showed that both the enzymatic (GST) and non-enzymatic (GSH) antioxidant defense systems were significantly decreased in the testes of rats, which is an indication that ACN may cause oxidative stress. Furthermore, measuring MDA generation revealed that ACN significantly increased lipid peroxidation. These results are consistent with ACN's shown capacity to cause oxidative stress in astrocytes in vitro and in vivo [37,38].

[39] Research has demonstrated that structurally related substances like dibromo acetonitrile (DBAN) significantly reduce the testicular content of GSH and increase the content of MDA when injected intraperitoneally (i.p.) into mice. There are two primary routes of ACN metabolism Direct synthesis of parent ACN and reduced glutathione (GSH) is required in the first pathway. N-acetyl-S (2-cyanoethyl) cysteine is formed and excreted in the urine as a result of the subsequent breakdown of this

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metabolite[40]. The second mechanism includes the epoxidation of ACN by cytochrome P450 2E1 (CYP 2E1), which produces the reactive and relatively long-lasting epoxide 2-cyanoethylene oxide (CEO) as an intermediate. CEO then makes hydrolysis or conjugation with GSH to produce cyanide (CN—) and other metabolites. By increasing lipid peroxidation and hydroperoxide formation, CN production causes oxidative stress. Additionally, the biological system's most significant enzyme, cytochrome oxidase, is inhibited by CN—, which results in the reduction of energy synthesis as seen by the decrease of ATP generation. [41].

Reactive oxygen species (OH., O.—, and H2O2) are produced by the hepatic microsomal enzymes cytochrome-P450 or peroxidases bioactivating cyanide-containing compounds (acrylonitrile, dibromoacetonitrile, and choloroacetonitrile), which releases CN ions that interact with specific compounds to produce free radicals [42]. Histological and ultrastructural defects indicate the testicular toxicity caused by ACN. Histopathology examination of the damaged tubules in the ACN- group demonstrated the production of multinucleated large cells, tubular atrophy, and loss of germ cells. [41]concluded that mice given ACN showed degradation of the germinal epithelium, seminiferous tubules, and reduced sperm count. In addition, the cessation of energy-dependent processes can make oxidative stress worse [43]. Thus, enzymatic conjugation and/or direct interaction with thiol groups might be responsible for the GSH-depleting effects of ACN, which in turn led to an increase in lipid peroxidation. In addition, oral administration of ACN to rats reduces sperm count and motility and inhibits the activity of pachytene antioxidant resveratrol exerted a protective role on Leydig cell steroidogenesis to produce testosterone; thus it stimulated the development of reproductive organs through the growth of Leydig and Sertoli cells and the promotion of spermatogenesis. Resveratrol treatment increased GSH level and also enhanced the activity of antioxidant enzymes SOD, CAT, GR, Gpx and GST in rats exposed to cypermethrin. In the present study, resveratrol treatment enhanced T, FSH and LH level. The increase in testicular T may be due to increase cell viability and testicular mass and synchronism in all reproductive parameters. The increase in T may be direct effect of resveratrol on Leydig cells and steroidogenesis. Increase in FSH and LH may be due to direct effect of resveratrol on hypothalamus-pituitary axis [44]. ACN intoxication also induced marked alterations in most of the seminiferous tubules including germ cell depletion, tubular atrophy, maturation arrest, complete necrosis as well as multinucleated giant cell formation. Expansion of intertubular spaces and interstitial

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haemorrhage were also illustrated[45]. previous reports have shown increased levels of FSH, LH, and testosterone in control rats after RES therapy [23]. Consistent with these findings, data of the current study show that levels of testosterone, FSH, and LH were significantly increased in the serum of control rats treated with RES ($p > 0.001$), decreased in the serum of Cis-treated rats ($p \leq 0.005$), and returned to their baseline levels in the serum of Cis + RES-treated rats, as compared to control rats (Figure 1A–C). The stimulatory effect of RES on HPG axis could be explained by the ability of RES to bind estrogen receptor (ER) as a mixed weak agonist/antagonist without estrogenic properties leading to hypophisary stimulation and HPG axis [46]. Previous researches reported that antioxidants suppress the generation of reactive oxygen species by different mechanisms including, blunting the oxide and hydrogen peroxide, scavenges lipid peroxidation products during free radical, suppresses excess NO production[47]. Free radicals, such as hydroxyl radicals and superoxide anions, have been related in the testicular toxicity of ACN in several studies[48]. [29]Recently, noticed that the mice testes significantly changed pathologically after administration of an intraperitoneal injection of dibromo acetonitrile. Multiple research studies indicate that ACN changes the DNA structure in testes, a condition that may significantly affect reproductive behavior. After a single oral dosage of ACN (46.5 mg/kg), [49]showed that covalent binding of ACN to testicular tissue DNA was seen in the testes of rats. At 0.5 hours following treatment, a significant decline in DNA synthesis (80% of control) was also seen. Testicular DNA synthesis decreased considerably (38% of control) 24 hours after ACN treatment. After administration with ACN, testicular DNA repair increased 1.5-fold at 0.5 h and by more than 3.3-fold at 24 h. These findings indicate that ACN has the potential to serve as a multi-potent genotoxic agent by alkylating DNA in testicular tissue and may have an impact on male reproductive function by obstructing testicular synthesis of DNA and repairing mechanism. [50]Demonstrated that the three resveratrol dimers, Par, Qua, and Pal, are all strong singlet oxygen scavengers that are selectivity. The two additional ROS, hydroxyl radical and superoxide anion, are little affected by their inhibitory actions. The present study indicates the beneficial effects of resveratrol against ACN- induced testicular toxicity. Resveratrol treatment improved the levels of endocrine parameters including T, FSH and LH. In the present study, resveratrol treatment enhanced T, FSH and LH level. The increase in testicular T may be due to increase cell viability and testicular mass and synchronism in all reproductive parameters. The increase in T may be direct effect of resveratrol on Leydig cells and

steroidogenesis. Increase in FSH and LH may be due to direct effect of resveratrol on hypothalamus-pituitary axis [43].

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