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Research Article

Acrylamide Induced Toxicity in Seminal Vesicles and its Amelioration by Vitamin E Supplementation: A Histological Study

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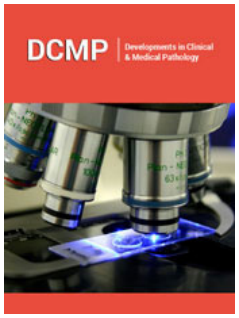

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Abstract

Acrylamide (ACR) is an odorless, water soluble vinyl monomer which exhibits several toxicological properties that affects almost all the major organ systems of human body. Reproductive toxicity due to ACR seems to be more pronounced in males than females. To elucidate the effect of ACR on reproductive system in males we designed the current study investigating the histopathological changes in seminal vesicles of male Wister rats. We also explored the ameliorative potential of vitamin E on toxicity induced by ACR in seminal vesicles. The experimental rats were divided into four groups. Group I served as control, Group II were treated with ACR, Group III with ACR+ vitamin E and Group IV was treated with only vitamin E. Our results clearly depicted noticeable changes in the histology of seminal vesicle of rats i.e., disorganization of mucosal folds with desquamation, necrosis of covering epithelium, cracked luminal secretions, loss of compactness of muscle layer and atrophied muscles. These changes were constructively reversed in Group III which was supplemented with vitamin E while ACR was also present. Distinguishable changes were observed in this group as evident by preservation of mucosal fold architecture and columnar covering epithelium of seminal vesicle. The muscle layer showed potential improvement in some rats but did not completely return to its normal compact appearance in others. Thus, our study elucidated the deleterious effect of ACR on seminal vesicles for which vitamin E acted as an ameliorative agent.

Introduction

Acrylamide (ACR) is a potent toxicant that is primarily released during industrial processes of polymer manufacturing due to hydration of acrylonitrile [1]. Another major source of ACR toxicity is its generation in some carbohydrate rich foods such as potatoes, breads and cookies that are cooked at high temperatures (120 °C or above) [2,3]. When these foods are subjected to extreme heat, a reaction between amino acids such as asparagine and reducing sugars such as glucose/fructose present in these foods occurs which causes the release of ACR (Maillard reaction) [3,4]. Whether being a dietary or occupational exposure, ACR is known to inflict serious toxicological effects in human body. Various studies in animal models have been conducted to mark the potential toxicological effects of ACR. ACR is profoundly reported to cause neurotoxicity, nephrotoxicity, carcinogenicity, genotoxicity, developmental anomalies, and reproductive defects [5-7]. The Scientific Committee on Toxicity, Ecotoxicity and the Environment (SCTEE) reports ACR to exhibit genotoxic effects in germ cells as well as in reproductive system of males as well as females [8]. Studies regarding reproductive toxicity of ACR have elucidated many histopathological changes in various organs and components of the reproductive system, especially in males. Some of the well observed anomalies caused by ACR in male reproductive system include testicular epithelial tissue degeneration, decrease in epididymal sperm reserves, histopathological lesions in testes, decrease in sperm count and motility and abnormalities in sperm morphology [5,9]. Even though numerous studies have pointed towards a deleterious effect of ACR on sperm production and morphology, none of them have explored the effect of direct ACR exposure on seminal vesicles [10,11]. Seminal vesicles are the glands that are partly responsible for production of the seminal fluid which

provides a thriving environment to the sperms. The underlying cause of toxicological alterations by ACR is the generation of oxidative stress that leads to changes of some major macromolecules such as proteins and DNA. However, further mechanisms that alter the functioning of reproductive system on exposure to ACR need to be investigated. Experimental studies in rats have shown that once ACR enters the body, it generates glycidamide, which is a more harmful metabolite than ACR itself [12]. This reaction is mediated by enzyme cytochrome P450 E1 (CYP2E1) [13]. Glycidamide is known to have a high potential for causing DNA as well as protein damage as compared to the unmetabolized ACR [14]. In the process of generation of glycidamide by CYP450 E1 from ACR, many free radicals are also released that consequently lead to a build-up of oxidative stress, causing lipid peroxidation and alterations in essential proteins required for normal metabolism and functioning of a cell [15].

Thus, considering the capability of ACR to generate oxidative stress, antioxidant compounds such as Vitamin E (alpha-tocopherol), could be explored as a potential ameliorative agent against ACR induced toxicity. Vitamin E or alpha-tocopherol is a type of fat-soluble vitamin found frequently in the cell membranes. It exhibits strong antioxidant properties which inhibit lipid peroxidation produced by the free superoxide and hydroxyl radicals [15]. Many studies have revealed the protective role of vitamin E in sperm cell against the damages of Reactive Oxygen Species (ROS) along with improvement in sperm motility as well as reproductive function [16,17]. As ACR is known to induce reproductive toxicity by affecting sperm morphology, motility or sperm count, we try to explore the cause of this alteration by conducting a histopathologic study of seminal vesicles on being exposed to ACR, since these glands play a major role in maintenance of sperm health. Following this we investigate vitamin E as a potential attenuating agent of ACR induced toxicity in seminal vesicles. To the best of our knowledge, this is the first study that directly investigates the effect of ACR on seminal vesicles and its amelioration by vitamin E.

Materials and Methods

Materials

The plus one acrylamide (PAGE) grade of >99.95 purity was procured from Pharmacia Biotech (Upsala, Swedan). Vitamin E (DL-α-tocopherol Acetate) was procured from Sigma-Aldrich (Steinheim-Germany). All other materials and chemicals used in the study of molecular biology grade were procured from BHD laboratory supplies (Analar®, England).

Animals and Treatment: Forty-nine adult male Wister rats were purchased from King Fahad Medical Research Centre (KFMRC), Jeddah, Kingdom of Saudi Arabia (KSA). All animal care procedure and treatments were carried out at KFMRC. On arrival these rats were 60 days old and weighed 250±20 g. Four rats were housed per polypropylene cage with bedding made of wood shavings. The environment around the rats was controlled throughout the experiment as relative humidity of 40-65%, temperature 22±2 °C and 12 hours/12 hours light/dark cycles. The rats were provided with adequate amount of tap water and were fed laboratory chow. The animals and study design were approved by the Unit of Biomedical Ethics, King Abdulaziz University (KAU), Medical College, Jeddah, KSA. The guidelines laid by KAU follow the national and international laws and policies (National Institutes of Health Guiding Principles on the Care and Use of Laboratory Animals, USA). The experimental rats were left to adapt to the environment for 3 days before initializing the dosing. The rats were clustered into four groups (n=7). Group I was of normal control rats which were provided with tapped water and laboratory chow during the complete experimental period. Using orogasteric needle, Group II rats were treated with only acrylamide (45mg/kg bw/day) by oral gavage which was selected as effective dose for inducing ACR toxicity in our other studies as well [10]. Group III rats were administered with both acrylamide + vitamin E (200mg/kg bw/day). Group IV was the drug control group for vitamin E treatment where the rats were given vitamin E alone (200mg/kg bw/day). This experiment was performed for 5 consecutive days. All rats were checked and weighed daily for any abnormal behavior or sudden death in experiment and recovery period. After a recovery period of 1-day post cessation of ACR the rats were sacrificed by cervical dislocation and both seminal vesicles were isolated for further experimental assessment.

Histopathology

Tissue preparation and histopathological examination: The section of seminal vesicle of all rats were isolated and fixed immediately by 10 % natural buffered formalin for 24 hrs. Tissues were then processed using automatic tissue processor (Shandon, England) by using standard laboratory procedures for histology. Tissues were briefly embedded in paraffin blocks, sectioned, and then stained with Hematoxylin and eosin stain (H&E). Tissues were examined for any histological changes using light microscopy (Olympus BX51TF) at 10X, 20X, 40X magnification and representative images were captured with Olympus DP 72 camera.

General observation: The Group II experimental rats that were treated with only acrylamide had a rough coat and showed signs of aggression and rough coat. The intake of food and water was also apparently reduced. Improved food and water intake were noticed in Group III which received a combined treatment of acrylamide with vitamin E. A normal food and water intake were also observed in Group IV rats who received only vitamin E. Throughout the experimental period, no symptoms of illness or mortality were observed in any of the groups (control as well as experimental).

Histopathology of seminal vesicle sections of control group and drug-control groups (Vit E alone): The control group which was fed only with laboratory chow and tap water during the experiment showed normal layers of seminal vesicle tissue, intact mucosal folds covered by columnar epithelium having highly basophilic and compact smooth muscle layers. The secretion within the lumen was observed to be homogenous and highly acidophilic (Figure 1a). Similarly, in the group treated with vit E alone, intact mucosal layers with normal epithelium could be seen. The muscular layers of these seminal vesicles were also observed to be well organized.

Effects of acrylamide on seminal vesicle (Group II): The Group II rats that were treated only with acrylamide showed visible changes in histopathological sections of seminal vesicle of experimental rats. Marked disorganization of mucosal folds with desquamation and necrosis of covering epithelium could be observed in different regions of seminal vesicle, the luminal secretions were noticed to be cracked. The examined muscle layer showed a loss of its compact structure, being atrophied, and appeared widely separated (Figure 2).

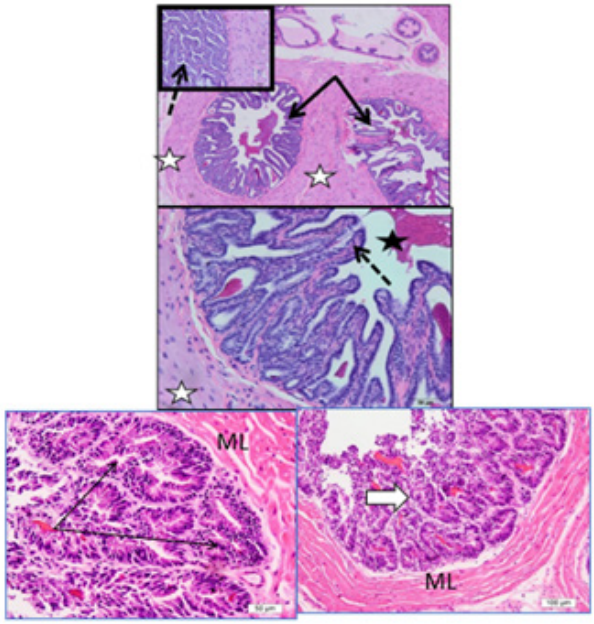

Figure 1: (a) The control group showing section of seminal vesicle stained with H and E stain. Black arrows indicate intact mucosal folds. Presence of highly basophilic columnar epithelium (dotted arrows). White stars indicate compact smooth muscle layers and black star indicates homogenous and highly acidophilic lumen secretion. **(b)** Group IV treated with vitamin E alone showing section of seminal vesicle stained with H and E stain. Low (x100x200) and high (x400) magnification photographs of rat seminal vesicle of Vitamin E group. White arrows indicate intact mucosal layers, ML indicates muscle layers and black arrows represent the lining epithelium.

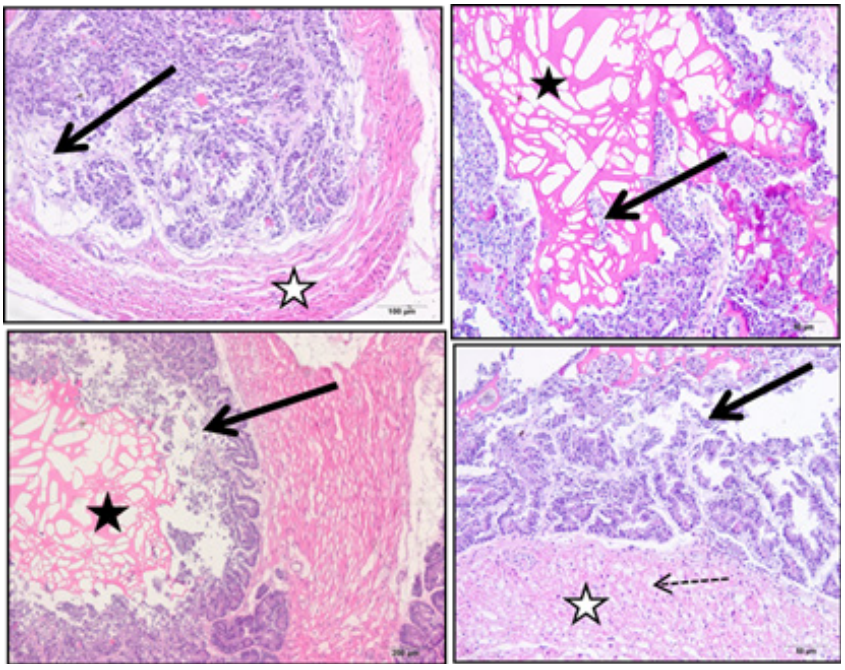

Figure 2: Group II treated with acrylamide showing section of seminal vesicle stained with H and E stain. Black arrows indicate disorganized mucosal folds with necrosis and desquamation of covering epithelium. Black stars indicate cracked luminal secretions. White star indicates widely separated and atrophied muscle layer with lose compact structure.

Effects of acrylamide + vitamin E on seminal vesicle (Group III): The Group III rats that were treated with acrylamide + vitamin E showed visible changes in sections of seminal vesicle of experimental rats. The results showed evident preservation of mucosal fold architecture and the covering columnar epithelium of seminal vesicle. The muscle layer showed potential improvement in some rats as compared to the rats receiving only ACR. However, on the other hand, the muscle layer of a few rats did not completely return to its normal compact appearance (Figure 3).

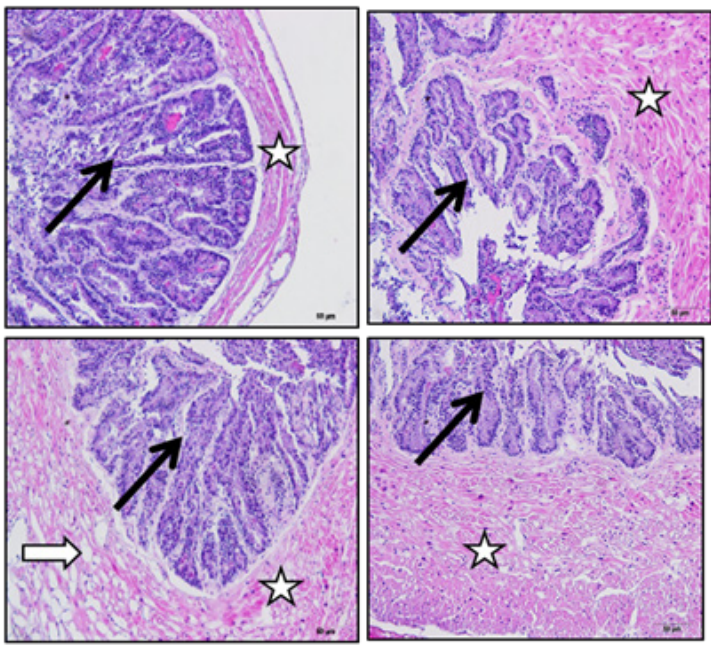

Figure 3: Group III treated with acrylamide + vitamin E showing section of seminal vesicle stained with H and E stain. Black arrows indicate preservation of mucosal fold architecture, columnar covering epithelium. White stars indicate potential improvement in muscle layer in some experimental rats. White arrow indicates incomplete return of muscle layer to its normal compact appearance in some experimental rats.

Discussion

Acrylamide (ACR) is an infamous toxicant known to have negative implications on most of the organ systems of a human body. It has been reported to act as neurotoxicant, reproductive toxicant and carcinogen in animal models. Monomeric ACR has been shown to cause cellular damage in both nervous and reproductive systems and tumors in specific hormonally responsive tissues [18]. Reproductive toxicity caused by ACR has been a subject of research dating long back. Reproductive toxicity due to ACR is found to have many serious implications, especially in males, primarily affecting the motility, count, and morphology of sperms [19]. However, the exact mechanism leading to this phenomenon still needs to be identified. Thus, this study aims at investigating the toxicity of ACR in seminal vesicles and the role of vitamin E in its mitigation. The present study revealed that rats treated with 45mg/kg bw/day of ACR for 5 consecutive days became aggressive and developed rough coat. Consequently, their food and water intake decreased. A similar behavior has been reported in our previous studies as well [7,12]. This study clearly shows that ACR causes reproductive toxicity that affects seminal vesicles as evident by visible changes in the vesicular sections of experimental rats. The histological examination of the covering epithelium showed marked disorganization of mucosal folds with desquamation and necrosis, even the muscle layer lost its compactness and showed signs of atrophy. Many studies have been conducted previously to investigate the effect of ACR on male reproductive system in rats, however, none of them have focused on the seminal vesicles [20-23]. A study by Al-Karim et al., showed that ACR in low dose can produce structural changes in reproductive system of both female and male rats. They reported damage of germ cell layers and DNA, histological changes leading to sperm deformity as well as a decrease in seminiferous tubules of male rats on exposure to ACR. Since the histological changes were found in many other reproductive organs as well, this could be correlated with our findings [24]. Similar findings were also reported where a damage at histological and ultrastructural level was observed because of ACR induced toxicity in testis of the rats in the form of degeneration of the tissue and arrested spermatogenesis. Decreased diameter of seminiferous tubules and decreased epithelial height were also reported, however, these changes were improved by vitamin E treatment [25].

The present study also revealed that the rats treated with both ACR and vitamin E for 5 consecutive days showed noticeable changes in their seminal vesicles as well. However, there was a pronounced restoration of mucosal fold architecture, covering columnar epithelium of seminal vesicles and muscle layer in contrary to the ones treated with ACR only. The rats of this experimental group (ACR + vit E) also showed improved food and water intake. These findings correlate with the results of the study carried out by Erdemli et al. They reported that damages indicated by histo-morphological changes in reproductive organs of rats were

less when ACR was administered in combination with vitamin E as compared to when ACR was administered alone thus, signifying the healing effect of vitamin E. Their observations included the restoration of seminiferous tubules organization, initiating maturation of germ layer and decline of interstitial oedema [26]. Thus, histopathological examination of seminal vesicles in our study clearly demonstrated that exposure of rats to ACR has significant adverse effects on their reproductive system, however, these undesirable changes could be ameliorated by administration of vitamin E. This damage by could be attributed to the oxidative stress generated by ACR along with an imbalance in oxidant/antioxidant ratio, generation of glycidamide, shift towards oxidants and lipid peroxidation [26]. Vitamin E is known to be a potent antioxidant that protects against oxidative stress by inhibiting propagation of ROS reactions and lipid peroxidation, which could be one of the reasons that it showed a significant protective effect against ACR toxicity on seminal vesicles in our study as well. Our observations fall in line with this underlying principle as well as with other studies reporting that ACR induced harmful effects on male reproductive organs have been considerably improved by use of vitamin E [27,28]. Numerous studies have reported the protective effect of vitamin E against ACR induced toxicity in various /other organs such as kidneys, brain, testis, skeletal muscles etc (26, 30-32). In addition to this, research has suggested that using vitamin E in rats exposed to toxins such as ACR may cause a reversal of the atrophies [7,24]. To the best of our knowledge, this is the first study that marks ACR as a potent toxin affecting seminal vesicles of rats with vitamin E acting as an ameliorative agent of such toxicity. In conclusion, this study states that reproductive toxicity due to ACR is well pronounced in seminal vesicles accompanied by many histopathological changes. These histopathological changes could be constructively reversed by vitamin E supplementation in most of the cases with just a few exceptions. Thus, vitamin E proved to be an effective ameliorative agent which can protect seminal vesicles from the deleterious effects of ACR toxicity. Further studies need to be carried out on a larger sample size investigating the mechanisms of ACR toxicity and its amelioration by vitamin E on a molecular level so that it could pave a way for utilization of vitamin E as a protective drug against ACR induced toxicity.

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