Research Article
AMERICAN JOURNAL OF PHARMACY AND HEALTH RESEARCH
www.ajphr.com
2016, Volume 4, Issue 11
ISSN: 2321–3647(online)

Histological Effect of 5-Aminosalicylic Acid and Vitamin-E on Acrylamide Induced Prostate Toxicity In Rat

Nisreen Abdullah Rajeh[1*], Wajnat Abdulmajed Hariri[1]
1.Department of Anatomy, Faculty of Medicine, King Abdulaziz University, Jeddah, Kingdom of Saudi Arabia

ABSTRACT

The aim of this work was to study the association between subacute acrylamide exposure and prostate toxicity in male rats; and to compare the effect of two known antioxidants: Vitamin-E and 5- aminosalicylic acid on the induced prostate toxicity in male rats. King Fahad Medical Research Centre, King Abdulaziz University, Jeddah, Saudi Arabia. A total of 49 adult wistar rats (250 ± 20g) of 60 days age were divided into seven groups (control, acrylamide alone, acrylamide + 5-aminosalicylic acid, acrylamide + Vitamin -E, acrylamide + 5-aminosalicylic acid + Vitamin-E, Vitamin-E alone, 5-aminosalicylic acid alone). After 5 days of acrylamide oral gavage, rats were observed for 24 hours and sacrificed. Histopathology for the prostate and testosterone hormone were carried out. No significant changes were observed in testosterone, lactate dehydrogenase serum level and rats' body weight. Rats treated with 5-aminosalicylic acid alone did not show any protection against acrylamide induced prostate toxicity. Further Vitamin-E alone did not show any protection against acrylamide induced prostate toxicity. Interestingly, injection of acrylamide treated rats with both Vitamin-E and 5-aminosalicylic acid concomitantly showed moderate improvement in the general histology of the prostate toxicity induced by acrylamide. Injection of acrylamide treated rats with 5-aminosalicylic acid or Vitamin-E alone did not show protective effect on acrylamide induced prostate toxicity on the level of prostate histology. However concomitant treatment of acrylamide treated rats with both antioxidants showed moderate improvement in general prostate histological structure.

Keywords: Acrylamide, 5-Aminosalicylic acid, Antioxidant, Prostate toxicity, Vitamin-E.

*Corresponding Author Email: nrajeh@kau.edu.sa, drnisreen73@gmail.com
Received 28September 2016, Accepted 21 October 2016

Please cite this article as: Rajeh NR *et al.*, Histological Effect of 5-Aminosalicylic Acid and Vitamin-E on Acrylamide Induced Prostate Toxicity In Rat. American Journal of Pharmacy & Health Research 2016.

INTRODUCTION

Acrylamide (ACR), a vinyle organic compound is highly soluble in water. Thus, it is easily absorbed and distributed inside the human body. ACR is a crucial industrial compound used in various manufacturing processes such as in the formation of several polymers e.g., polyacrylamide. Polyacrylamide is mainly used in water treatment, paper and plastic industry, mineral production and electrophoresis in laboratory analysis.[1] The structure of acrylamide is shown in Figure 1.[2] The International Agency for Research on Cancer *(IARC*) has classified ACR *as* "a potential carcinogen to human". Furthermore, it is a neuro-reproductive toxicant in a wide variety of laboratory animals.[3] ACR is also a food toxicant, highly formed in carbohydrate rich food such as fried potato, coffee, cookies and breakfast cereals when exposed to high temperature during cooking in which Maillard reaction occurs between asparagine amino acids and glucose, producing acrylamide.[4,5]

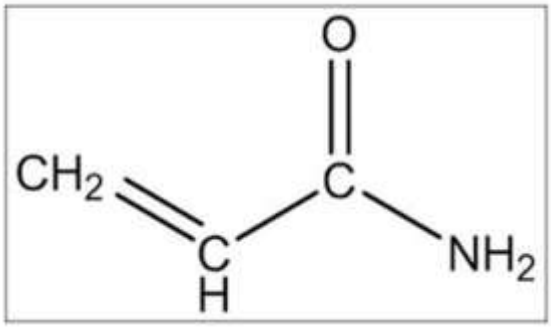

Figure 1: Structural formula of acrylamide.

Two major pathways known for ACR metabolism are; glutathione conjugation or glycidamide epoxidation.[3] The reactive toxic metabolite of ACR, known as glycidamide is more toxic towards proteins and DNA than acrylamide.[4] Cytochrome P450 E1 (CYP2E1) is the main enzyme involved in glycidamide epoxidation.[3,6,7] ACR metabolism by CYP2E1 in the liver mainly, causes release of free radicals that typically affect the oxidative defence system, leading to genotoxic and carcinogenic toxicity.[3]

Most of the literature reviews are about acrylamide and prostate cancer, and no results were matching toxicity only. Numerous prospective studies have found no evidence between ACR intake and high risk of prostate cancer.[5,8] In a recent study conducted by Rajeh et al.[3], ACR was reported to affect the epididymal sperm count and the serum testosterone level. In addition, ACR induced histological changes in the testes, including leydig cells and germ cell degeneration with disruption in the typical looking appearance of the testis.[3]

Antioxidants, *such as* Vitamin-E and 5-amino salicylic acid (5-ASA) are the major defence mechanism against oxidative stress caused by reactive oxygen species (ROS) generation induced by ACR.[9] Vitamin-E is lipid soluble compound; its most active biological form is α-tocopherol which prevents lipid peroxidation by supplying hydrogen atom to ROS instead of polyunsaturated fatty acids present in lipid membrane.[9] Potential studies have shown that prolonged period of supplementation using α-tocopherol significantly reduces chance of prostate cancer.[10] 5-ASA is reported to have antioxidant and anti-inflammatory role, which strongly protects liver from ROS mediated damage.[3] In addition, 5-ASA has therapeutic role in bowel inflammatory disease such as ulcerative colitis, which mostly depends on its antioxidant and anti-inflammatory properties.[11]

Significant reduction in serum testosterone concentration was induced after acrylamide treatment with a dose of 45mg/kg bw for 5 days.[3] Since prostate is known to be testosterone dependent gland, so detection of some pathologic changes after ACR treatment is expected.[3] However, the research on toxic effect of acrylamide on prostate toxicity is little and moreover, the antidote for this toxicity is not well studied. In this work, we tried to explore more toxic effects of acrylamide on prostate and compare the effects of two known antioxidants (Vitamin-E and 5-ASA) on the induced toxicity in male rats.

MATERIALS AND METHOD

Materials

Plus one acrylamide (PAGE) grade with purity >99.95 was purchased from Pharmacia Biotech (Uppsala, Sweden). 5-ASA 95%, Vitamin-E (DL-α-tocopherol Acetate), and > 98% HPLC (High Performance Liquid Chromatography) were purchased from (Sigma-Aldrich, Steinheim-Germany). Testosterone kit (ALPCO Diagnostics, Windham, USA) and other chemicals and materials of molecular biology grade (BHD laboratory supplies, Analar®, England) were used in carrying out the study.

Methods

Animals and Treatment

A total of 49 adult male virgin Wister rats (250±20 g), 60 days old on arrival, were purchased from King Fahad Medical Research Centre (KFMRC), Jeddah, Kingdom of Saudi Arabia (KSA) and were housed four per polycarbonate cage with wood shavings as bedding. These animals were maintained in controlled environment of temperature 22 ± 2 °C, relative humidity of 40 -

65% and 12 hours / 12 hours light / dark cycles throughout the experiment. The rats were fed laboratory chow and were supplied with ad libitum water.

All animal care procedure and treatments were carried out at KFMRC, Jeddah, KSA with the approval of the Unit of Biomedical Ethics, King Abdulaziz University (KAU), Medical College, Jeddah, KSA in accordance with the guidelines of the KAU. These guidelines are in compliance with the national and international laws and policies (National Institutes of Health Guiding Principles on the Care and Use of Laboratory Animals, USA).

Animals were allowed to acclimatize at the experimental environment for 3 days before dosage initiation. The rats were divided into 7 groups (n=7). One control group, four acrylamide treated groups (ACR alone, ACR+5-ASA, ACR+Vitamin-E and ACR+5-ASA+Vitamin-E) and the last two groups are for Vitamin-E alone and 5-ASA alone. The dose of acrylamide was prepared by using distilled water and used was 45 mg/kg/day for 5 consecutive days.[3] This ACR dose was administered by oral gavage using metallic needle curved-ball ended (Size PS-18). Control group was gavaged with 1 ml of distilled water. Rats were treated with Vitamin-E at a dose of 200 mg/kg/day by oral gavage according to Takhshid MA. et al.[12]. 5-ASA treated rats were intra-peritoneally injected with a dose (25 mg/kg/day) of 5-ASA for 5 consecutive days.[3] After 5 days of treatment, blood was collected from retro-orbital sinus of the left eye. After a recovery period of one day of ACR cessation, animals were killed by cervical dislocation and prostate gland of all rats were isolated for further experimental evaluation.

Histopathology

Prostate of all rats were fixed by 10% natural buffered formalin for 24 hrs.

Processing of Fixed Sections

Following fixation of tissues by previous methodology, tissues were then processed using standard laboratory procedures for histology. Tissues were briefly embedded in paraffin blocks, sectioned at approximately 3-5 µm thickness and then stained with haematoxylin and eosin (H and E). Slides were examined for histological changes using light microscopy (Olympus BX51TF) at 10X, 20X, 40X magnification and representative images were captured with Olympus DP 72 camera.

Biochemical Analysis

Testosterone (Enzyme Linked Immunosorbent Assay)

Blood of forty-nine male Wister rats was collected in plain tubes of red top caps to obtain their serum for promoting testosterone hormonal analysis by means of an automated analysis administration using ADVIA Centaur and ADVIA Centaur XP Systems (Siemens Healthcare

Diagnostics) for quantitative and competitive immunoassay. The correlation coefficient (r=0.99 at 1% level of significance or 5 % level of significance) indicated the inverse relation between testosterone levels and the amount of light unit (RLUs) detected by the system.

Lactate Dehydrogenase Assay (LDH) Assay

Lactate dehydrogenase (LDH) is commonly found in the cytoplasm within different mammalian bodies and can be easily evaluated by using quantitative data measurements obtained by Dimension Vista® System and Flex® reagent cartridge. The reaction took place within 96 micro-well plate where all reagents are ready to use liquid solutions.

Statistical Analysis

All statistical analysis was done using SPSS (Statistical package for the Social Sciences) 16.0 software (SPSS Inc., Chicago, IL, USA). Data was expressed as mean ± 2SD. Differences among the groups were analysed by one-way analysis of variance (ANOVA) followed by the Tukey's test as a post hoc for multiple comparisons. A P-value of less than 0.05 was considered as criterion for a statistically significant difference.

RESULTS AND DISCUSSION

General Observation

Rats treated with a dose of 45 mg/kg/day ACR showed signs of aggression and rough coat, with reduction in food and water intake. Improvement in water and food intake was detected in group treated with ACR and 5-ASA. Rats in the control group showed no symptoms of illness during the experimental period. No mortality was recorded among all the seven groups.

Effect of Acrylamide and Antioxidants on Body Weight Changes of Rats

Administration of acrylamide to rats at a dose of 45 mg/kg/day for 5 consecutive days did not show any significant difference in body weight change between groups, one day after cessation of ACR treatment (Figure 2). Body weight changes at the end of observation periods were calculated. Similarly, rats which were orally gavaged with Vitamin-E (200 mg/kg/day), and injected intraperitoneally (IP) with 5-ASA (25mg/kg/day) with gum acacia as a solvent, concomitantly with oral ACR treatment, did not show any change in body weight. Data were expressed as mean ± 2SD (twice the value of standard deviation), n = 7. No statistically significant difference was detected between groups.

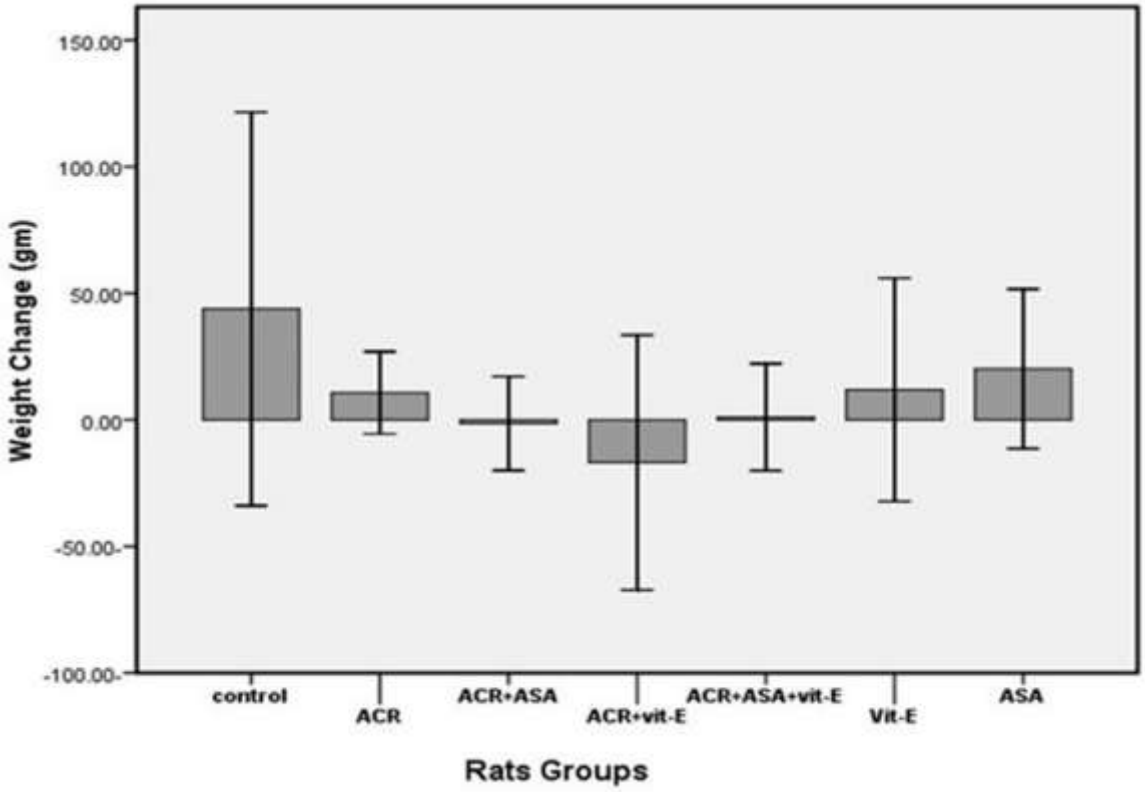

Figure 2: Effect of acrylamide (ACR) and antioxidants on rats' body weight changes at the end of observation period.

Effect of ACR and Antioxidants on Serum Testosterone Level

As testosterone is produced within the leydig cells of the testis, we next examined the impact of ACR mediated toxicity on circulating testosterone levels and the protective ability of 5-ASA and Vitamin-E against ACR toxicity (Figure 3). The results showed no statistically significant difference between groups.

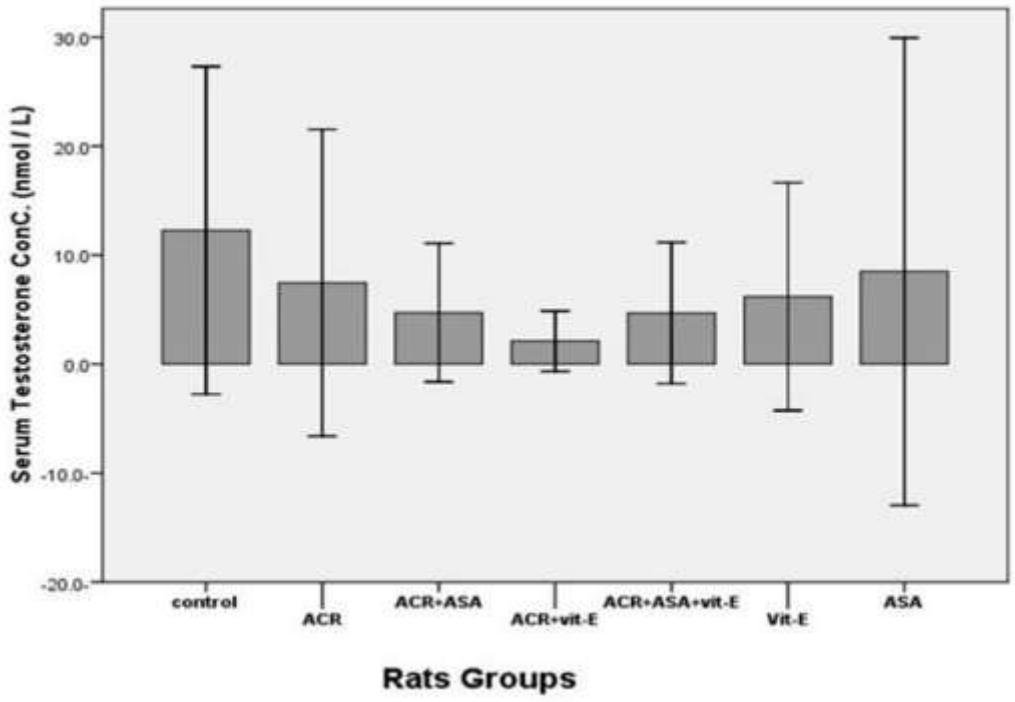

Figure 3: Effect of acrylamide (ACR) and antioxidants on serum testosterone concentration of rats.

Effect on Serum Lactate Dehydrogenase (LDH) Concentration

In this study, no significant difference ($P > 0.05$) of lactate dehydrogenase serum concentration was detected in ACR treated or any antioxidant treated group of rats (Figure 4).

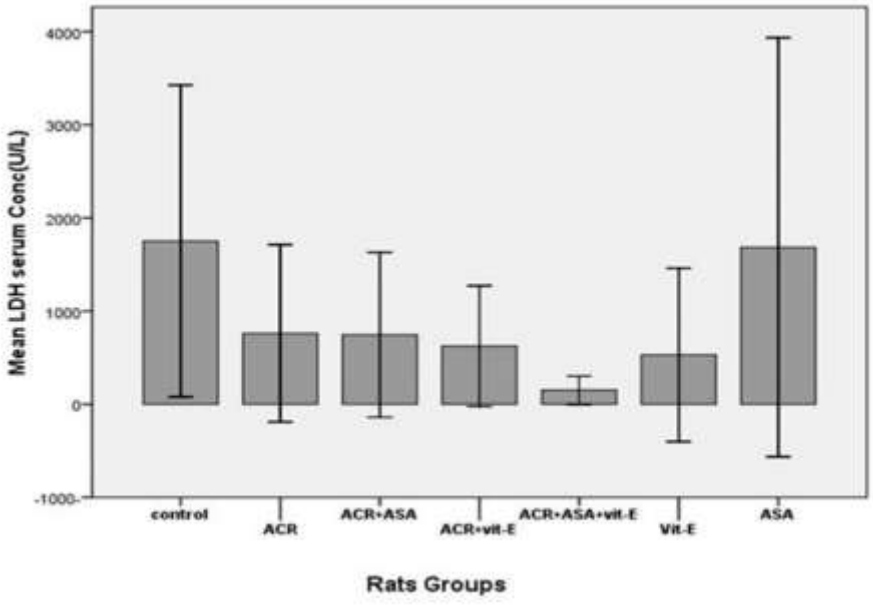

Figure 4: Effect of acrylamide (ACR) and antioxidants (Vitamin-E and 5-ASA) on serum lactate dehydrogenase (LDH) of rats.

Effect on Prostate Histology

Some evidences of histological changes in the prostate were found in rats treated with ACR when compared with the control. In control (Figure 5A) prostatic acini appeared normal with different sizes of normal defined basement membrane, normal general cellular arrangement with apparent luminal acidophilic secretions and normal appearance of infolded mucosa of the acini. The prostatic acini are also separated by narrow intercellular spaces occupied by minimal stroma (Figure 5A,5B,5C).

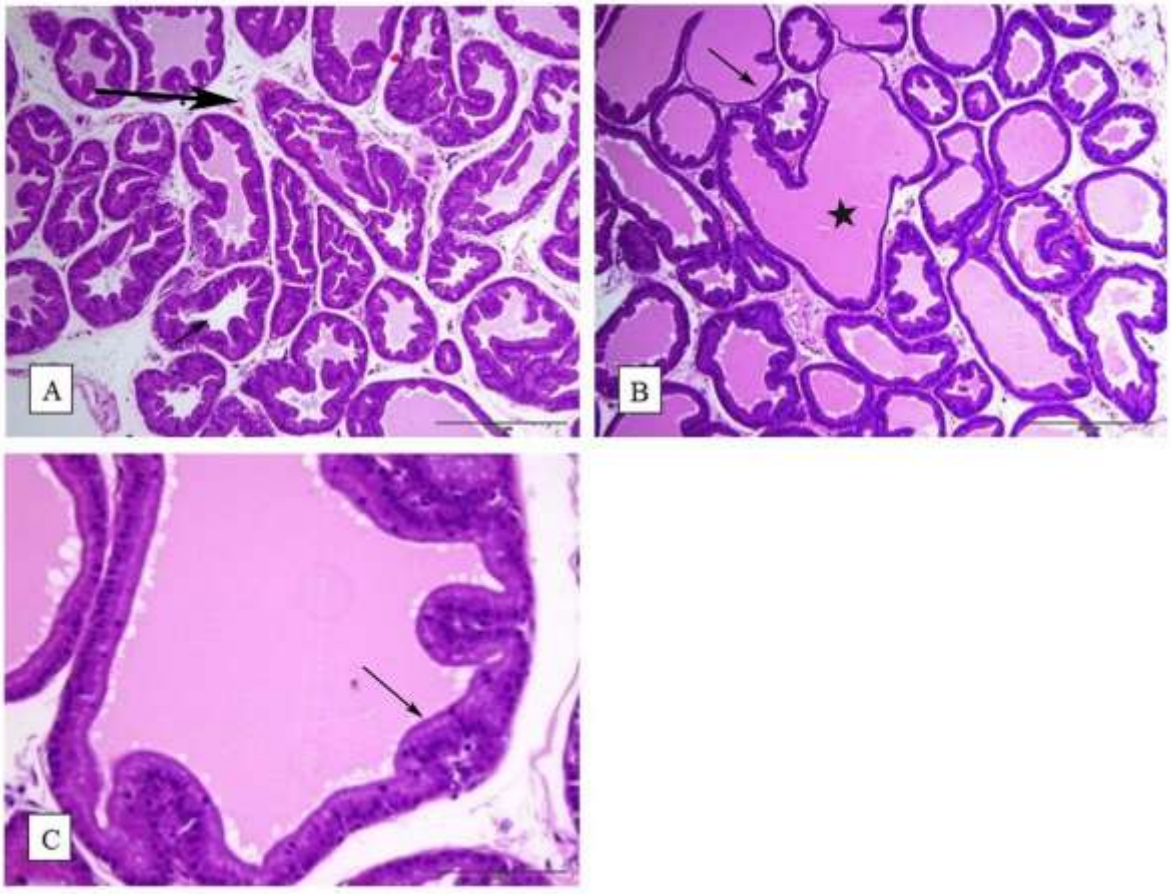

Figure 5: Light microscopy of transverse sections of prostate isolated from control adult Wister rat. (A) Shows prostatic acini that are closely packed of different sizes and infolded mucosa (arrows), these acini are separated by narrow interacinar spaces occupied by minimal stroma (long arrow), 10X magnification; **(B)** Acini of different sizes contain homogenous acidophilic secretion (asterisk) with normal height of lining columnar epithelium; **(C)** Shows magnified acinus lined with normal columnar epithelium (arrow), 40X magnification. Sections were stained with H & E stain and viewed with light microscopy.

However, rats gavaged with a dose 45 mg/kg/day ACR for 5 days (Figure 6), appeared to have some pathological features including; dilated thin–walled prostatic acini in most of the ACR treated rats with no apparent effect on prostatic secretion (Figure 6A). Also, decrease in normal mucosal infoldings of the acini was noted in this group with no apparent effect on acini number. In addition, reduction in the height of epithelial lining of some acini with flattened acinar cells was noted. Arterial congestion was noted in the interacinar space (Figure 6B). No inflammatory cellular infiltration was noted. Apparent reduction in stromal tissue with widening of interacinous spaces of the prostate gland was observed.

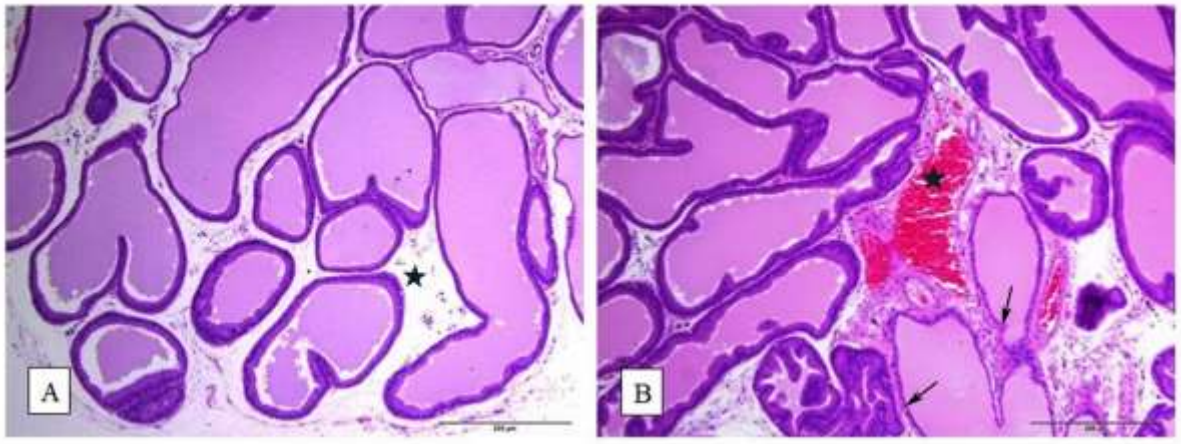

Figure 6: Light microscopy of transverse sections of prostate isolated from acrylamide treated rats (acrylamide was given at a dose of 45 mg/kg). (A) Shows increase in interacinous space with decreased stromal tissue (thinning of stroma) (asterisk) and widening of acini with apparent secretion in the lumen of acini, 10X magnification; **(B)** Shows interacinar arterial congestion with flattened acinar cells (arrow), 10X magnification. Sections were stained with H&E stain and viewed with light microscopy.

In the current study injection of ACR treated rats with 5-ASA did not show any protective effect on the prostate. Histopathological changes noted were in the form of flattened acini, increased congestion in interacinar space and decreased stroma (Figure 7). Furthermore, rats treated with ACR+Vitamin-E did not show any protection against ACR toxicity. Histological changes that took place include decrease in normal mucosal infoldings or flattened acinar cells. Also, congestion of interacinar vessels was also found (Figure 8).

Interestingly, injection of ACR treated rats with Vitamin-E and 5-ASA concomitantly for 5 consecutive days induced moderate improvement in the general histology of the prostate when compared to the treatment of 5-ASA or Vitamin-E alone. It was noted that there is neither increase in interacinar space nor effect on interacinar stroma with minimal congestion. Abundant

prostatic secretions with minimal flattening of acinar cells were also noted (Figure 9A, 9B). Animal control groups treated with Vitamin-E and 5-ASA alone did not show major pathology with normal looking appearance of infoldings and stromal tissue (Figure 10).

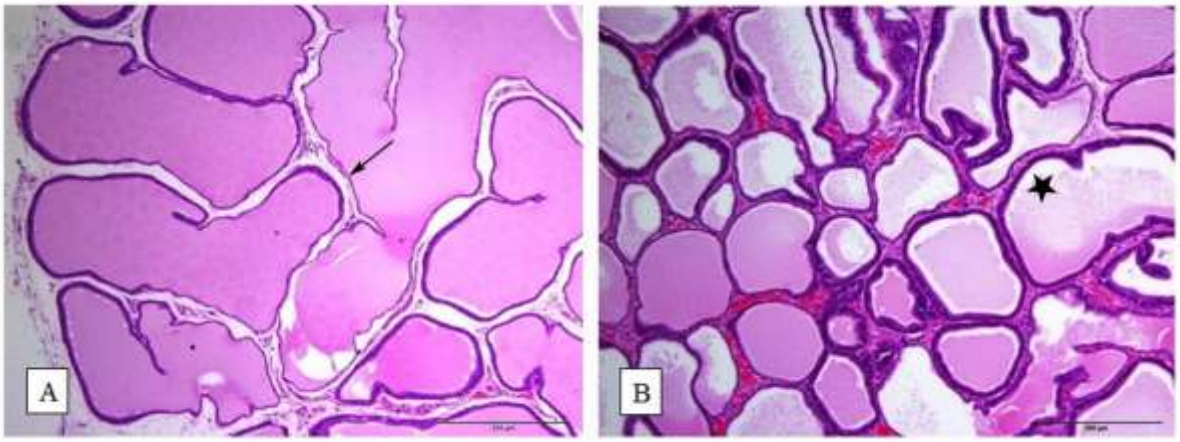

Figure 7: Light microscopy of transverse sections of prostate isolated from acrylamide + 5-aminosalicylic acid treated rats. (A) Shows dilatation and flattened acini (arrow); **(B)** Shows congestion in interacinar space with reduction in secretions (asterisk). Sections were stained with H & E stain and viewed with light microscopy at 10X magnification.

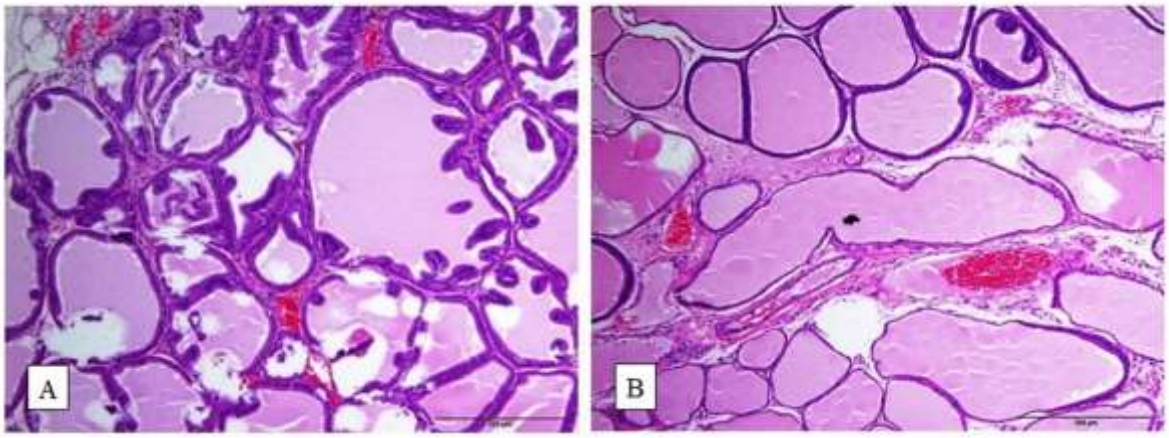

Figure 8: Light microscopy of transverse sections of prostate isolated from acrylamide + Vitamin-E treated rats. (A) Shows decreased infoldings of acini; **(B)** Shows congestion of vessels in interacinar space. Sections were stained with H & E stain and viewed with light microscopy at 10X magnification.

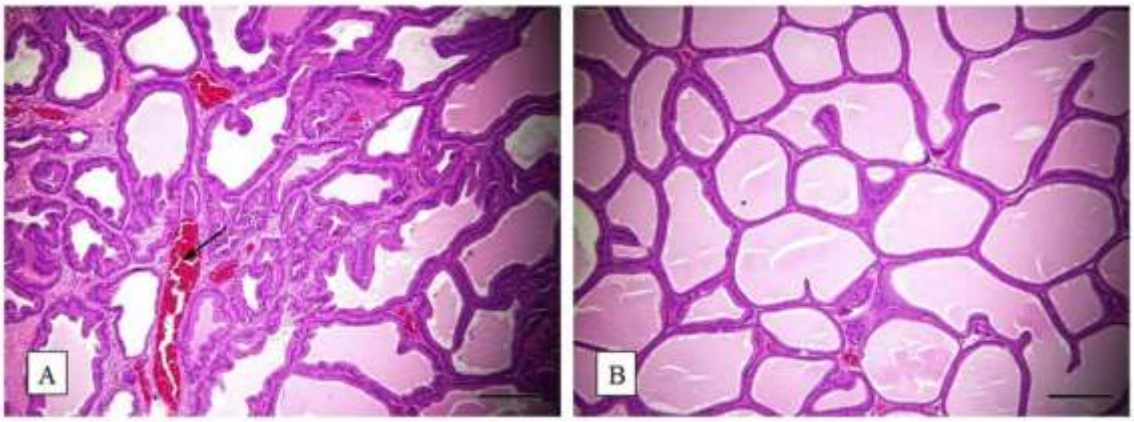

Figure 9: Light microscopy of transverse sections of prostate isolated from rats treated with acrylamide + Vitamin-E+ 5-aminosalicylic acid. (A) Shows vascular congestion in interacinar space (arrow); **(B)** Shows acini with decreased infoldings and no effect on interacinar space with normal looking stroma. Sections were stained with H & E stain and viewed with light microscopy at 10X magnification.

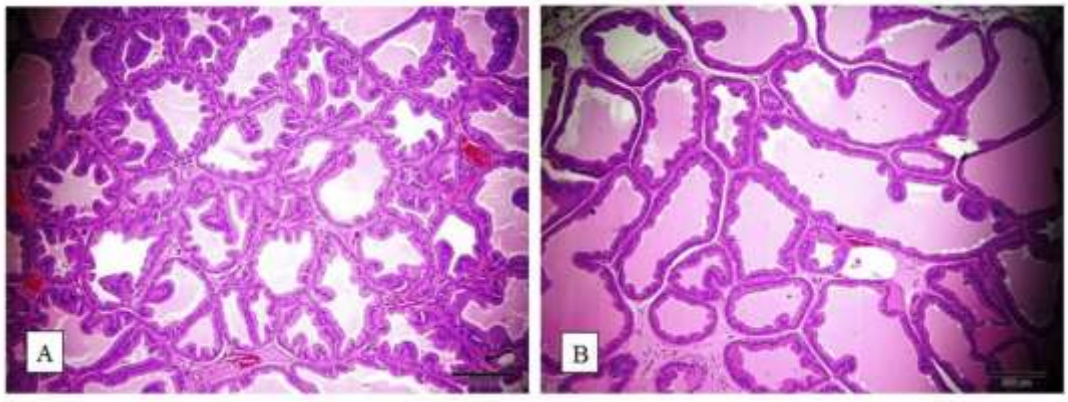

Figure 10: Light microscopy of transverse sections of prostate isolated from rats treated with (A) Vitamin-E alone and (B) 5-ASA alone. Shows good mucosal infoldings with normal stroma and interacinar space. Sections were stained with H & E stain and viewed with light microscopy at 10X magnification.

DISCUSSION

Acrylamide is recognised worldwide as an important environmental contaminant and food toxicant. Many studies investigated the association between dietary acrylamide exposure and prostate cancer risk, but studies on prostate toxicity were not done.[5,8] Hence, we examined ACR toxicity on rat prostate after subacute exposure to ACR for a duration of 5 days on a high dose of 45 mg/kg/day to induce prostate toxicity and to investigate the protective effect of 5-ASA and Vitamin-E on the induced toxicity.

In the current study, we found that ACR caused prostate toxicity that might improve histologically by concomitant injection of 5-ASA and Vitamin-E for 5 consecutive days. ACR administration to rats at a dose of 45 mg/kg/day for 5 consecutive days did not show any significant difference in body weight change between tested groups. This result was in consistency with other study reported by Lafferty et al.[13] in which ACR treatment did not show any significant difference in rats' body weights. The current finding could be attributed to short-term exposure to ACR and also the type of rats' strain used, moreover to the used dose of ACR.

As testosterone is produced within the Leydig cells of the testis and prostate gland is testosterone dependent gland, we next examined the impact of ACR-mediated toxicity on circulating testosterone levels and the ability of 5-ASA and Vitamin-E to protect against ACR toxicity. The result of this study showed no statistically significant difference among all the groups. This finding was on contrary to Rajah et al.[3] and Yang et al.[14], they reported statistically significant reduction in serum testosterone concentration after ACR treatment in comparison to control group. This variation can be attributed to observed period for one day only after ACR cessation.

In this study, no significant difference ($P > 0.05$) was detected among all groups regarding lactate dehydrogenase serum concentration. One study reported by Yousef M.I. and El-Demerdash F.M.[15], in which treatment of rats with the different concentrations of ACR (0.5, 5, 25, 50, 250 and 500 g/kg) for 10 weeks did not cause any significant changes in the activity of LDH compared to control group. These findings were similar to those observed in our study. On contrary to our result, one study conducted by Abd El-Mottaleb and Rashed.[16] on Albino rat showed significant increase in serum LDH concentration after 28 days of exposure in a dose (150mg/l) equivalent to 1/10 of LD (lethal dose). The current finding could be attributed to short period of exposure as LDH increase in serum is indicating the affection of cell membrane which will lead to leakage of many cellular enzymes as LDH, in which it is mainly present in heart, liver and kidney.[17]

In the current study, ACR produced pathological changes in the prostate rat in the form of flattened acinar cells and arterial congestion in the interacinar space. Most of the literature reviews were about acrylamide and prostate cancer but no results were matching toxicity only. Much prospective study found no evidence between ACR intake and increased risk of prostate cancer. Our explanation for the pathological features seen in prostate gland after ACR exposure is mostly due to reduction in testosterone hormone caused by acrylamide toxicity as detected in many previous studies.[3]

CONCLUSION

In conclusion, the injection of ACR treated rats with 5-ASA and Vitamin-E alone did not show any protective effect against ACR induced prostate toxicity. Still the ACR treated rats when administered concomitantly with both antioxidants i.e., 5-ASA and Vitamin-E confirmed moderate improvement in the general histology of the prostate. Therefore, we advocate restraint of ACR exposure either occupationally or in food products. Further investigations are required to ravel out and understand the molecular basis behind ACR prostate toxicity.

ACKNOWLEDGEMENTS

We thank Biochemical Ethics Unit of King Abdulaziz University, Medical College, Jeddah, KSA for approving use of animals and experimental design. We also acknowledge King Fahad Medical Research Centre (KFMRC), Jeddah, KSA for providing us the laboratory space for conducting experiments.

REFERENCES

1. Taeymans D, Wood J, Ashby P, Blank I, Studer A, Stadler RH, *et al*. A review of acrylamide: an industry perspective on research, analysis, formation and control. Crit Rev Food Sci Nutr 2004;44(5): 323-47.
2. Paulsson B, Rannug A, Henderson AP, Golding BT, Tronqvist M, Warholm M. In vitro studies of the influence of glutathione transferase and epoxide hydrolase on the detoxification of acrylamide and glycidamide in blood. Mutat Res 2005;580(1-2):53-59.
3. Rajeh N, Ali H, Elassouli S. Protective effect of 5-ASA on acrylamide toxicity in the testis and blood leukocytes of the rat. Kuwait Med J 2014;46(1):32-43.
4. Ghorbel I, Maktouf S, Kallel C, Chaabouni SE, Boudawara T, Zeghal N. Disruption of erythrocyte antioxidant defense system, hematological parameters, induction of pro-inflammatory cytokines and DNA damage in liver of co-exposed rats to aluminium and acrylamide. Chem Biol Interact 2015;236: 31-40.
5. Wilson KM, Giovannucci E, Stampfer MJ, Mucci LA. Dietary acrylamide and risk of prostate cancer. Int J Cancer 2012;131(2):479-87.
6. Islam MR, Parvin MS, Islam ME. Antioxidant and hepatoprotective activity of an ethanol extract of Syzygium jambos (L.) leaves**.** Drug Discov Ther 2012;6(4):205-11.
7. Kehrer JP, Jones DP, Lemasters JJ, Farber JL, Jaeschke H. Mechanisms of hypoxic cell injury: Summary of the symposium presented at the 1990 Annual Meeting of the Society of Toxicology. Toxicol Appl Pharm 1990;106(2):165-78.

8. Hogervorst JG, Schouten LJ, Konings EJ, Goldbohm RA, van den Brandt PA. Dietary acrylamide intake and the risk of renal cell, bladder and prostate cancer. Am J Clin Nutr 2008;87(5):1428-38.
9. Siahkoohi S, Anvari**1** M, Tafti MA, Sharifabad MH. The effects of vitamin E on the liver integrity of mice fed with acrylamide diet. Iran J Pathol 2014;9(2):89-98.
10. Heinonen OP, Albanes D, Virtamo J, Taylor PR, Huttunen JK, Hartman AM, *et al.* Prostate cancer and supplementation with alpha-tocopherol and beta-carotene: incidence and mortality in a controlled trial. J Natl Cancer Inst 1998;90(6):440-46.
11. Pearson DC, Jourd'heuil D, Meddings JB. The anti-oxidant properties of 5-aminosalicylic acid. Free Radic Biol Med 1996;21(3):367-73.
12. Takhshid MA, Tavasuli AR, Heidary Y, Keshavarz M, Kargar H. Protective effect of vitamins- E and C on endosulfan-induced reproductive toxicity in male rats. Iran J Med Sci 2012;37(3):173-80.
13. Lafferty JS, Kamendulis LM, Kaster J, Jiang J, Klauning JE. Subchronic acrylamide treatment induces a tissue-specific increase in DNA synthesis in the rat. Toxicol Lett 2004;154(1-2):95-103.
14. Yang HJ, Lee SH, Jin Y, Choi JH, Han DU, Chae C, *et al.* Toxicological effects of acrylamide on rat testicular gene expression profile. Reprod Toxicol 2005;19(4):527-34.
15. Yousef MI, El-Demerdash FM. Acrylamide-induced oxidative stress and biochemical perturbations in rats. Toxicology 2006;219(1-3):133-41.
16. Abd El-Mottaleb EM, Rashed A YM. Some studies on acrylamide intoxication in male albino rats. Egypt J Comp Path & Clinic Path 2008;21(4):222-45.
17. Sonnenwirth AC, Jarett L. Gradwohl's Clinical Laboratory Methods and Diagnosis. *C V Mosby* Washington 1980;1(8):258-59.