L-cysteine ameliorated testicular toxicity induced by acrylamide in rats

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ABSTRACT

The general public is exposed to acrylamide from industrial manufacturing, laboratory work, foods rich in carbohydrates that have been cooked at high-temperature, and through cigarette smoke. The present experiment was conducted to investigate the reproductive toxicity of acrylamide exposure in male rats and the role of L-cysteine supplementation in amelioration of this toxicity. Forty eight adult male albino rats (weighing 120-140 g) were divided into four groups (16 rats/group). Group I - negative control group, drank tap water, group II - positive control, drank tap water that contains acrylamide (25 mg/kg body weight) for 28 days and group III drank tap water that contains acrylamide (25 mg/kg body weight) and L-cysteine (100 mg/kg body weight). Four rats from each group were killed at 7, 14, 21 and 28 days time intervals from the beginning of the experiment. However, plasma testosterone was significantly decreased in acrylamide treated rats with congestion and interstitial edema, necrosis, calcification and degeneration of spermatogenic cells in the seminiferous tubules and formation of spermatid giant cells. Co-treatment of rats with L-cysteine reduced the changes in oxidative stress parameters and improved the pathological changes in testis. Therefore, supplementation of L-cysteine can be useful when there is a risk of acrylamide toxicity.

Keywords: Acrylamide, Testis, Oxidative stress, L-cysteine, Testosterone.

1. INTRODUCTION

Acrylamide (ACR) is formed through the Maillard reaction during the heating process by interactions of amino acids, especially asparagine, with reducing sugars like glucose [1, 2]. There is variance in literature about the levels of ACR in different foods and the potential risk from dietary exposure. The daily intakes of dietary ACR for the
general population are estimated to be in the range of 0.3-2.0 mg/kg body weight [3]. ACR can be reactive in three different ways, radical-mediated polymerization, and addition to thiol, hydroxyl, or amino groups result in alkylation of proteins or metabolized to an epoxide derivative, glyciamide being readily reactive toward DNA and other macromolecules [4-6]. The mechanism by which ACR exposure causes cellular dysfunction in experimental animals and humans is not completely clear. However, it is thought that oxidative stress was associated with ACR cytotoxicity. Reproductive toxicity in rodents exposed to ACR includes alterations in gonadal and pituitary hormones associated with histopathological changes that includes formation of multinucleated giant cells, vacuolation and production of high numbers of apoptotic cells in the seminiferous tubules [7-11]. Acrylamide induced oxidative stress is capable of disrupting the steroidogenic capacity of Leydig cells [12] as well as the capacity of the germinal epithelium to differentiate into normal spermatozoa [13]. Fortunately, testes contain a complicated group of antioxidants and free radical scavengers to protect the spermatogenic and steroidogenic functions of testis from oxidative stress [14].

Kurebayashi and Ohno [15] reported that glutathione (GSH) precursors such as N-acetyl-L-cysteine and L-methionine increased the protection against the cytotoxicity of ACR in isolated rat hepatocytes. Moreover, N-acetyl-L-cysteine as potent antioxidant protects tissues from ACR toxicity by inhibiting neutrophil infiltration, balancing the oxidant-antioxidant status, and regulating the generation of inflammatory mediators [8]. Also, α-lipoic acid protect cells from oxidative stress induced by ACR exposure via enhances cellular antioxidant defense capacity [9]. In this regard, the objectives of the present study were to measures the markers of oxidative stress in testis of rats exposed to ACR and to evaluate the protective role of L-cysteine as precursor of GSH against ACR toxicity.

2. MATERIALS AND METHODS

Forty eight adult male albino rats (weighing 120-140 g) were purchased from the Animal House, Faculty of Medicine, Assiut University, Assiut, Egypt. The animals were housed in cages at a controlled temperature (25±3°C) and ambient humidity (50-60%). Lights were maintained on a 12-h light-dark cycle. All animals received basal diet and water ad-libitum for one week as an adaptation period. Following one week of acclimatization, the rats were randomly divided into three groups (16 rats/group):

- Group I: Negative control, fed on basal diet and normal drinking water for 4 weeks.
- Group II: Positive control, fed on basal diet and drinking water that contains ACR (25 mg/kg body weight) according to Alturfan et al. [8].
- Group III: Fed on basal diet and drinking water that contains ACR (25 mg/kg body weight and L-cysteine (100 mg/kg body weight) according to Omar et al. [16].

Then, each week from the beginning of the experiment 4 rats from each group were killed under anesthesia with ether. The blood samples were collected directly from portal vein into centrifuge tubes for separation of serum by centrifugation at 3000 rpm for 15 minutes and were frozen at -20°C for subsequent biochemical analysis. Immediately after killing rats, small piece of testes were excised and fixed in formaline for histological studies, and the remnant was washed in cold saline, immersed in liquid nitrogen and stored at -20°C for biochemical assay. All animal experiments were carried out in accordance with Ethical Committee Acts.

2.1. Determination of oxidative stress biomarker

Lipid peroxidation (LPO) products as TBARS content were determined according to the method of Ohkawa et al. [17]. Nitric oxide (NO) content was measured as nitrate concentration colorimetrically using the method of Ding et al. [18]. Glutathione (GSH) content was determined using the method of Beutler et al. [19]. The activity of superoxide dismutase (SOD) was determined basing on its ability to inhibit the autoxidation of epinephrine at alkaline medium according to the method of Misra and Fridovich [20]. The activity of catalase (CAT) was determined basing on its ability to decompose H$_2$O$_2$ to H$_2$O and O$_2$ according to Gregory and Fridovich [21]. Protein content in the spleen tissues was determined by the method of Lowry et al. [22].
2.2. Estimation of testosterone

Testosterone hormone in plasma was determine by Enzyme Immunoassay Method (ELISA), Biocheck, Inc, 323 vintage Park Dr. Forster City, CA, USA, according to the kit manufacture instructions.

2.3. Statistical analysis

The data was expressed as mean ± SE. The results were analyzed statistically using column statistics and one-way analysis of variance with the Newman-Keuls multiple comparison test as a post-test. These analyses were carried out using the computer prism program for windows, version 6.0 (Graph pad software Inc., San Diego, California, USA). Differences between the groups were considered significant if $P < 0.05$, 0.01, or 0.001.

3. RESULTS

Compared to control rats, ACR treated rats exhibited a significant decrease in plasma testosterone in 2\textsuperscript{nd}, 3\textsuperscript{rd}, and 4\textsuperscript{th} weeks and LC treatment resulted in an increase in testosterone level especially in 2\textsuperscript{nd} and 4\textsuperscript{th} week (Fig. 1).

In relation to control rats, ACR treated rats had greater level of LPO in testis especially in the 3\textsuperscript{rd} week ($P<0.001$) and LC co-treatment failed to restore the elevation of LPO especially in 1\textsuperscript{st} and 4\textsuperscript{th} week (Fig. 2). Also, Fig. 2 showed that NO was significantly increased ($P<0.001$) in all periods of the experiment in comparison with control and LC treatment resulted in significant reduction in NO level ($P<0.001$).

In the ACR group, GSH level in testis was decreased significantly ($P<0.001$) in comparison to the control group. In the LC + ACR group, GSH level was resorted to the normal level in control group (Fig. 3). Moreover, SOD and CAT activities were significantly increased ($P<0.05$) in the 3\textsuperscript{rd} week in ACR treated rats, while they significantly decreased ($P<0.001$) in the same week in LC and ACR treated groups (Fig. 3).

3.1. Histopathology of testes

In the present study, it was observed that testis of control rats stained with hematoxylin and eosin (H&E) was formed of seminiferous tubules and interstitial cells of Leydig. Most tubules contain normal spermatogenic cell layers and spermatzoa (Fig. 4A). After one week of treatment with ACR, testes showed mild degeneration of spermatogenic cells in the seminiferous tubules, interstitial edema and degeneration in spermatogenic cells with formation of spermatid giant cells (arrows) (Fig. 4B). After two weeks, the testes showed severe degeneration in the spermatogenic cells with the presence of spermatid giant cells, the absence of
some spermatogenic cells layers and the presence of spermatid giant cells in some cases was also noticed (Fig. 4D). After three weeks, testes showed severe coagulative necrosis of seminiferous tubules and the absence of spermatogenic cells (Fig. 4F). And after four weeks, testes showed severe necrosis with dystrophic calcification in the seminiferous tubules (Fig. 4H). In group of rats co-treated with LC for one week, testes showed congestion, interstitial edema and hyperplasia Leydig cells in the first week and mild degeneration in the spermatogenic cells (Fig. 4C). After 2 weeks of treatment with LC, testes showed mild degeneration of the spermatogenic cells with the presence of multiple spermatid giant cells (Fig. 4E). In rats co-treated with LC for 3 and 4 weeks, testes showed mild degeneration in some seminiferous tubules (Figs. 4G and I).

4. DISCUSSION

ACR is one of the major environmental public health problems; it induced oxidative organ damages in the brain, lung, liver, kidney, spleen, and testes tissues [8, 23-25]. The present result in (Fig. 2) revealed that administration of ACR in drinking water increased LPO and NO in testes and co-treatment of rats with LC decreased the level of LPO and NO in comparison with ACR treated rats. It is known that, NO may show either cytoprotective or cytotoxic effects and the elevated NO level has the ability to induce γ-glutamyl cycle [26]. In consistence with our results, Jiazhong et al. [27] and Abd El-Halim and Mohamed [23] mentioned that ACR is able to increase LPO by inducing oxidative stress with generation of free radicals. Garlic, resveratrol and NAC may be protected against ACR-induced oxidative injury by scavenging free radicals, preventing depletion of GSH and inhibiting neutrophil infiltration, and subsequent activation of inflammatory mediators that induce LPO [8, 28].

ACR is capable of interacting with vital cellular nucleophiles possessing -SH group. Therefore, it reacts with GSH in a similar manner and forms GSH S-conjugates, which is the initial step in the biotransformation of ACR [29]. Depletion of cellular GSH seems to play a pivotal role in the genotoxicity in human caused by ACR [30, 31]. In the present study, decreased GSH content in testes as presented in (Fig. 3) can be explained by the reaction of ACR with GSH, which in turn causes the depletion of GSH and the enhancement of LPO. Similarly, Abd El-Halim and Mohamed [23] found that administration of ACR caused a significant reduction in testes GSH level. However, Özturan-Özeri et al. [32] concluded that GSH is not directly capable of protecting tissues against ACR-induced oxidative stress. The present results showed that co-treatment of rats with LC attenuate GSH depletion by ACR. In consistence, treatment with garlic prior to ACR attenuated the depletion of GSH level [18]. Also, treatment of rats with lipoic acid caused an increase in GSH level with a decrease in LPO which might be attributed to the oxidative damage repairing ability of lipoic acid [33].
Superoxide radical may oxidize SH groups and undergo dismutation to form $\text{H}_2\text{O}_2$ and singlet oxygen [34]. This change in the redox status of the cell may modulate gene expression directly or via the transcription factors that are redox-regulated, and may lead to apoptosis, cell proliferation, or transformation (29). In the current study, (Fig. 3) showed alteration in the testicular SOD and CAT activities depending on the period of treatments. Also, it showed that co-treatments of rats with LC ameliorate these changes by different levels. These results are in agreement with Abd El-Halim and Mohamed [23] who found that administration of ACR caused a significant reduction in the activity of SOD in testes tissues. The reduction in antioxidant enzyme activities was increased with increasing doses of ACR [35]. Treatment with *Curcuma longa* L. powder and garlic prior to ACR attenuated the reduction of SOD activity [23, 36], and administration of catechin and neem leaves extracts significantly enhanced the hepatic CAT activity [37].

Testosterone level in plasma of rats treated with ACR was significantly decreased, and co-treatment with LC elevates this decrease especially in the 4th week of treatment (Fig. 1). In this aspect, administration of ACR caused a significant reduction of serum testosterone level as reported by many authors [9, 38, 39]. This significant reduction of testosterone may be a result of direct damage of ACR on the Leydig cells [40]. The previous opinion was confirmed histopathologically in the present study.
study by congestion and interstitial edema, necrosis, calcification and degeneration of spermatogenic cells in the seminiferous tubules with formation of spermatid giant cells (Fig. 4 A, C, E & G). Moreover, ACR may affect the endocrine function of the testes by altering the androgen biosynthesis of interstitial cells in the testes [41] or inducing the enzymes activity of hepatic biotransformation, which is capable of metabolically transforming androgens into products with low androgen receptor binding activity [42]. Song et al. [43] found that ACR can directly damage Leydig cells and affect the endocrine function of the testis. Moreover, Yang et al. [44] found that ACR induces histopathological lesions such as formation of multinucleated giant cells, vacuolation and production of high numbers of apoptotic cells in the seminiferous tubules of the rat. In the present study, treatment with LC along with ACR resulted in moderate attenuation of the histopathological changes in testes that were induced by ACR.

From these observations, it can be concluded that LC ameliorates the toxicity of ACR in rat testes by alleviating LPO and NO through scavenging of free radicals, and enhancing the activity of SOD and CAT and GSH level.

AUTHORS CONTRIBUTION

All authors contributed equally in planning, conduct, data analysis, and editing the work. The final manuscript has been read and approved by all authors.

TRANSPARENCY DECLARATION

The author declares no conflicts of interest.

NOTES

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REFERENCES


