Research Article

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Biomarkers Journal
ISSN 2472-1646

2015
Vol. 1 No. 1:5

DOI: 10.21767/2472-1646.100005

Acrylamide Induced Testicular Toxicity in Rats: Protective Effect of Garlic Oil

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Citation: Elghaffar SKA, Fiedan IO, Ahmed EA, et al. Acrylamide Induced Testicular Toxicity in Rats: Protective Effect of Garlic Oil. Biomark J. 2016, 1:1.

Abstract

Objective: Acrylamide (ACR) is prominent in fried, baked and heat- processed starchy foods. The present experiment was conducted to investigate the testicular toxicity of oral acrylamide exposure in male rats and the role of garlic oil in amelioration of this toxicity.

Method: Forty-eight adult male Sprague-Dawely albino rats (weighing 120-140 g) were divided into three groups (16 rats/ group); group I: negative control group, tap water for 28 days; group II: positive control, tap water containing ACR (25 mg/ kg body weight) for 28 days; and group III: tap water containing ACR (25 mg/kg body weight) and treated twice a week for 4 consecutive weeks with garlic oil (50 mg/kg body weight). Four rats were killed at 7, 14, 21 and 28 days time intervals from beginning of experiment.

Results: The administration of ACR resulted in elevation in testes malondialdehyde (MDA) and nitric oxide (NO) levels with significant reduction in the level of glutathione (GSH) and the activity of superoxide dismutase (SOD) and catalase (CAT) in all periods of the experiment. Also, plasma testosterone was significantly elevated in ACR- treated rats as compared with the negative control. These biochemical changes were associated with congestion, interstitial edema, degeneration of spermatogenic cells in the seminiferous tubules, and formation of spermatid giant cells as well as necrosis and calcification. Treatment with garlic oil alongside ACR ameliorates this biochemical and histopathological changes.

Conclusion: Supplementation of garlic oil, which is a sulfur containing compound act as a substrate for GSH antioxidant, may offer protection against ACR induced testicular toxicity.

Keywords: Acrylamide; Garlic oil; Rats; Testis; Testosterone; Oxidative stress; Histopathology

Received: November 14, 2015; **Accepted:** December 01, 2015; **Published:** December 07, 2015

Introduction

Acrylamide (ACR) is an important chemical that is used in various scientific and industrial processes, such as water treatment, cosmetics, and gel electrophoresis. Also, ACR is found in carbohydrate rich foods that have been cooked at high temperatures, and in tobacco smoke. Lifelong exposure to ACR at low intake levels through food and smoking has potential health effects [1-4]. Research has been reported the toxic effect of ACR on nervous [5], reproductive system [6], and skeletal muscle [7]. This toxicity of ACR is related to its affinity for sulfhydryl groups on proteins which could inactive proteins/enzymes involved in DNA repair [8, 9]. Moreover, ACR itself, but not oxidative P450 metabolites appear to be involved in ACR-induced cellular transformation [10]. For example, testes of rats treated with ACR showed necrosis of epithelial cells lining the seminifrous tubules, as well as degeneration and necrosis of the Lydig cells [11]. Moreover, ACR affect oocyte quality through reactive oxygen species (ROS) generation and induction of apoptosis [12].

Medicinal plants and natural herbal products are used as chemotherapeutic agents to provide protection against toxic side effects due to their antioxidant activity [13, 14]. Garlic is one the medicinal plants commonly worldwide used food and

due to its medical properties [15]. Garlic and its organosulfur compounds, mainly S-Allylcysteine and allicin have been reported to have diverse biological activities such as anticarcinogenic, anti-artherosclerotic, antidiabetic, renoprotective, antioxidant and immune modulation, antibacterial, antihypertensive and various other biological actions. The organosulfur compounds of garlic exert their antioxidant actions by scavenging ROS, enhancing cellular antioxidant enzymes and increasing glutathione in the cells [16-18].

The present study, designed to support the role of oxidative stress in ACR toxicity and to elucidate whether supplementation with garlic oil as antioxidant could modulate ACR-induced testicular damage in rats.

Materials and Method

Forty eight adult male albino rats (weighing 120-140 gm) were purchased from the Animal House, Faculty of Medicine, Assiut University, Assiut, Egypt. All animal procedures were performed in accordance Declaration of Helsinki with the guidelines for the care and use of experimental animals that was established by the Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the National Institute of Health (NIH) protocol**.** 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. [19].
- Group III: Fed on basal diet and drinking water that contains ACR (25 mg/kg body weight and garlic oil (100 mg/kg body weight) according to Meki and Omar [20].

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 formalin for histological studies. Other specimens from the testes were 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.

Determination of oxidative stress biomarker

Lipid peroxidation (LPO) products as thiobarbituric reactive substances (TBARS) content were determined according to the method of Ohkawa *et al*. [21]. Nitric Oxide (NO) content was measured as nitrate concentration colorimetrically using the method of Ding *et al*. [22]. Glutathione (GSH) content was determined using the method of Beutler *et al*. [23]. 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 [24]. The activity of catalase (CAT) was determined basing on its ability to decompose H_2O_2 to H_2O and O_2 according to Gregory and Fridovich [25]. Protein content in the spleen tissues was determined by the method of Lowry *et al*. [26].

Estimation of testosterone

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

Prepartion of tissues for histopathological observation

The 10% neutral buffered formalin fixed testis were routinely processed according to standard procedures. Then, sections (7 µm) of the different groups were mounted on slides and dried overnight at 37°C. The sections were de-waxed in xylene, hydrated in a graded series of alcohol solutions and then stained with hematoxylin and eosin for histological evaluation according to Drury and Wallington [27]**.**

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

Results

Histopathological results

Examination of H&E stained sections of the testis of the control group showed normal histological structure in which most seminiferous tubules contain normal spermatogenic cells layers and spermatozoa **(Figure 1A)**. In ACR treated groups, after two weeks, the testes of showed degeneration of spermatogenic cells with formation of spermatid giant cells in the seminiferous tubules **(Figure 1B)**. Severe degeneration in the spermatogenic cells with only presence of some spermatogonia and sertoli cells were seen after three weeks **(Figure 1C)**. At the end of the experiment, the testes showed severe coagulative necrosis of seminiferous tubules with absence of spermatogenic cells **(Figure 1D)**. Testis of ACR plus garlic oil treated groups at different weeks of treatment showed mild degeneration in some seminiferous tubules with interstitial edema and hyperplasia of Leydig cells **(Figure 1E and 1F)**.

Biochemical results

In **Figure 2** the result of the current study showed a statistically significant decrease in testosterone in rats exposed to ACR for

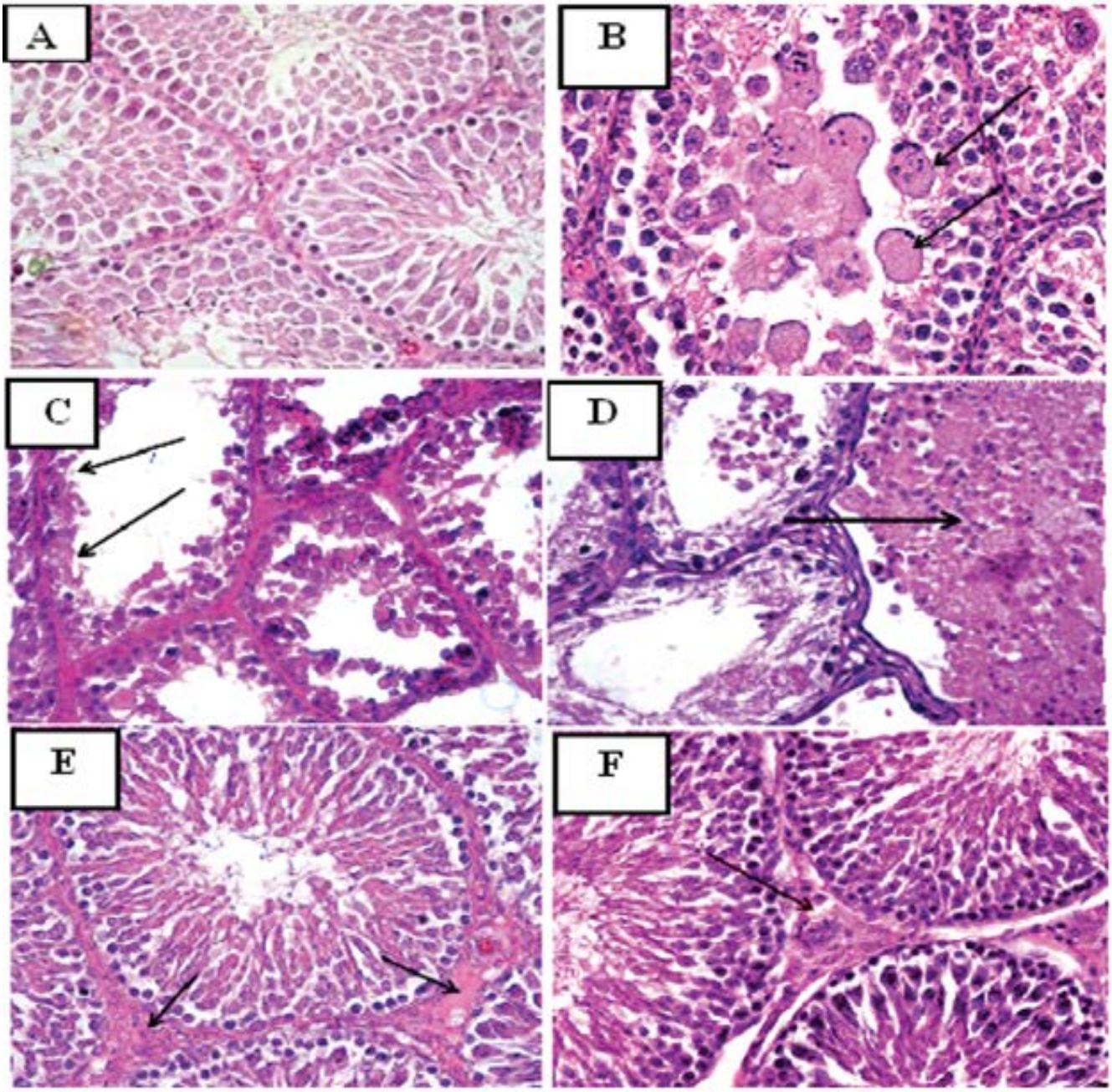


Figure 1 Photomicrographs of testis form control and treated rats. A. Most tubules contain normal spermatogenic cells layers and spermatozoa in control rats. B-D testis of Acrylamid treated groups at different weeks of treatment. **B**. mild degeneration of spermatogenic cells with formation of spermatid giant cells in the seminiferous tubules (arrows). **C**. Severe degeneration in the spermatogenic cells, only some spermatogonia and sertoli cells could be seen (arrows). **D.** Severe coagulative necrosis of seminiferous tubules and absence of spermatogenic cells (arrows). E-F testis of Acrylamid plus garlic oil treated groups at different weeks of treatment showing mild degeneration in some seminiferous tubules with interstitial edema and hyperplasia of Leydig cells (arrows). **H&E X400**

2, 3 and 4 weeks than the control group while, there was non-statistically significant increase in rats exposed for one week. Moreover, co-treatment of rats with garlic oil failed to restore the level of testosterone to the control.

Figure 3 showed the level of LPO and NO in the testicular tissue. LPO was significantly increased in testis of rats exposed to ACR for 1 and 3 weeks, however non significantly increased in testis of rats exposed to ACR for 2 and 4 weeks in comparison with the control. Also, **Figure 2** showed that co-treatment of rats with garlic oil caused an increase in LPO in testis of rats exposed to ACR for 1 and 4 weeks, however, LPO level was decreased in testis of rats exposed to ACR for 2 and 3 weeks.

Moreover, **(Figure 3)** showed that NO was significantly increased in testicular tissue of rats exposed to ACR in comparison to control and co-treatment of rats with garlic oil restored the NO level to the control except in rats exposed to ACR for 2 weeks.

(Figure 4) showed that SOD activity was decreased in testis of rats exposed to ACR for 1 and 3 weeks, however was increased in 2 and 4 weeks in comparison with control. Co-treatment of rats with garlic oil results in a decrease in SOD activity in testis of rats exposed to ACR for the last 3 successive weeks in comparison to ACR treated groups. The activity of CAT **(Figure 4)** was increased in the first 3 weeks of experiment in ACR and ACR with garlic oil treated groups in comparison with control, however in the 4th week was similar to the control group. The content of GSH in testicular tissue was significantly decreased in all periods of experiment in comparison with control group and co-treatment of rats with garlic oil results in an increase in GSH content in testis of rats exposed to ACR for 2 and 3 weeks in comparison with ACR treated rats **(Figure 4)**.

Discussion

Testis is a target organ of ACR action as it caused severe damage in seminiferous tubules and caused decrease in plasma free and total testosterone in a dose dependent manner [28]. In the present study testosterone level in sera of rats treated with ACR significantly decreased, and co-treatment with garlic oil elevate this decrease especially in 3rd and 4th week of treatments **(Figure 2)**. This result is in consistence, other authors found that administration of ACR caused a significant reduction of serum testosterone level [11**,** 29, 30]. This significant reduction of testosterone may be a result from the direct damage of ACR to the Leydig cells [31-33]. Moreover, ACR may alter the androgen biosynthesis of interstitial cells in the testes [34] or induces the enzymes activity of hepatic biotransformation, which is capable of metabolically transforming androgens into products with low androgen receptor binding activity [35]. The reduction in serum testosterone was accompanied by the histopathologicaly changes that represented by congestion and interstitial edema, necrosis, calcification and degeneration of spermatogenic cells in the seminiferous tubules with formation of spermatid gaint cells **(Figures 1B-1E)**. Similar observation was reported by Yang et al. [32] who found that ACR induces histopathological lesions such as formation of multinucleated giant cells and production of high numbers of apoptotic cells in the seminiferous tubules of the rat. In the present study, treatment with garlic oil along with ACR resulted in moderate attenuation of the histopathological changes in testes that induced by ACR **(Figure 1F)**.

The present result in **Figure 3** revealed that administration of ACR in drinking water increased testicular LPO as expressed by an increase in TBARS level. Similarly, Jiazhong et al. [36] Abd El-Halim and Mohamed [37] found that administration of ACR caused a significant elevation in testes MDA level. Also, **Figure 3** showed that co-treatment of rats with garlic oil decreased the level of LPO in comparison with ACR treated rats. In comparison, garlic, resveratrol and N-acetyl cysteine protected against ACR-induced oxidative injury by scavenging free radicals and inhibiting neutrophil infiltration, and subsequent activation of inflammatory mediators that induce LPO [19, 38**].** Nitric oxide levels, in terms of total NO_2 concentration in testes was increased in testicular tissue **(Figure 3)**. This elevation of NO level may induce the γ-glutamyl cycle which prevent GSH depletion [39]. Because, ACR is able to interact with the vital cellular nucleophiles possessing-SH group and GSH to forms GSH S- conjugates, which is the initial step in the biotransformation of ACR [40]. So, depletion of cellular GSH may play a critical role in the genotoxicity of ACR [41, 42]. In the present study, decreased GSH content in testes as presented in **Figure 4** can be explained by the reaction of ACR with GSH, which in turn causes the depletion of GSH and the enhancement of LPO. In consistence, Abd El-Halim and Mohamed [37] found that administration of ACR caused a significant reduction in testes GSH

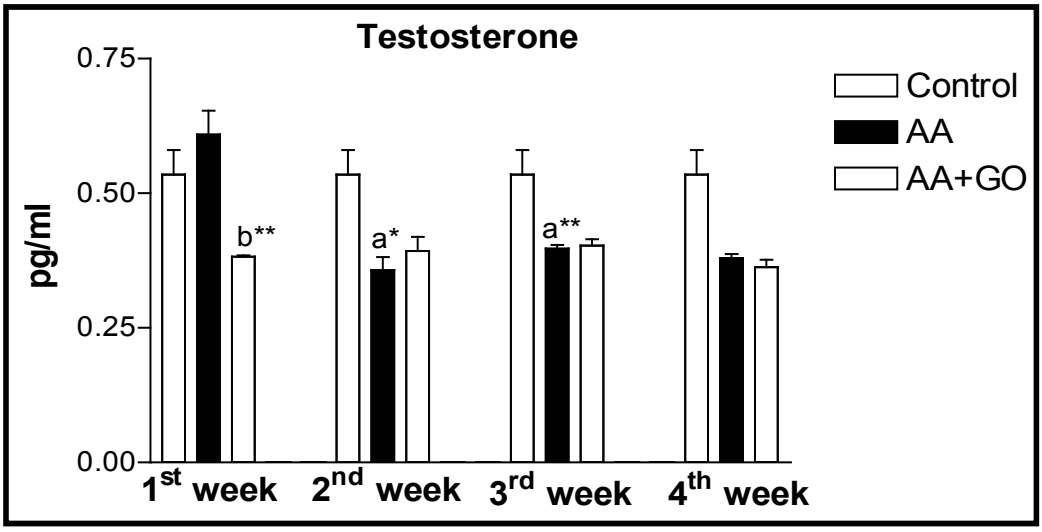
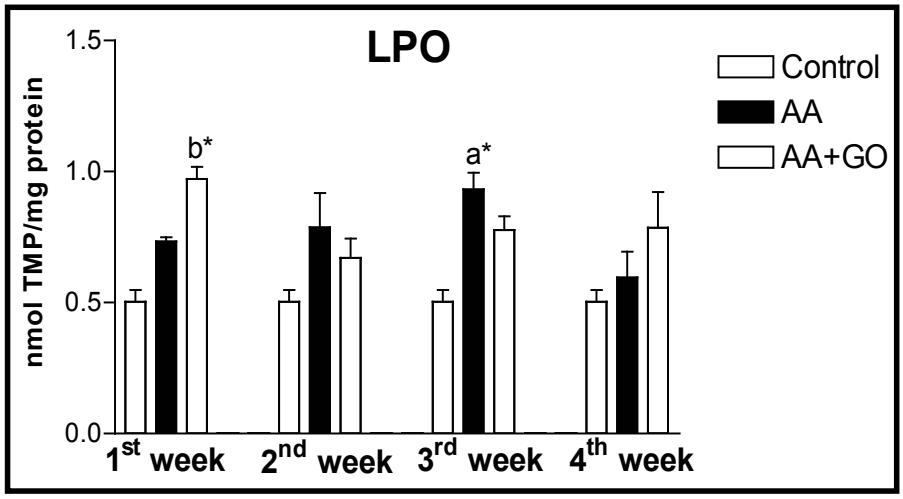


Figure 2 Level of testosterone in sera of control and treated rats at different time intervals of ACR exposure
Letter [a] indicates a significant differences between ACR and control groups (* *P*<0.05 & ** P<0.01)
Letter [b] indicates a significant differences between ACR plus garlic oil and ACR groups (* *P*<0.05 & ** P<0.01)



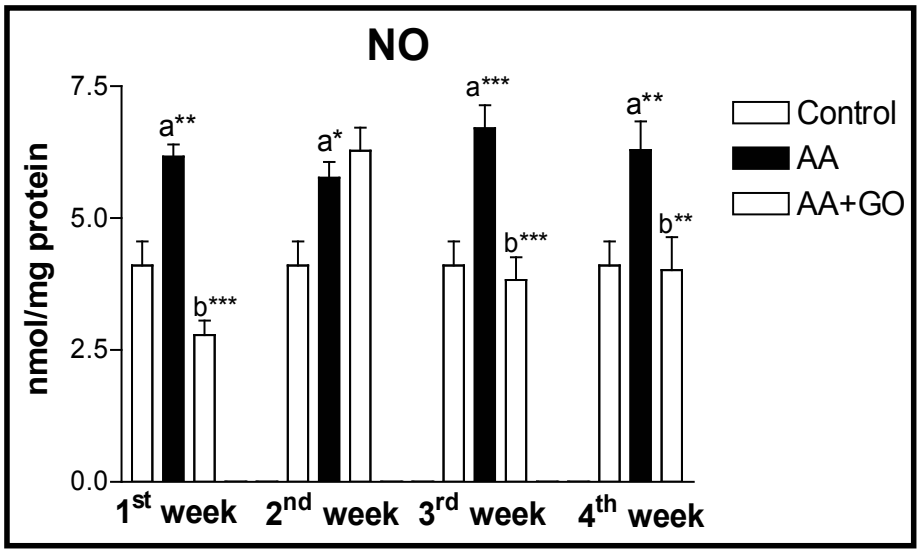
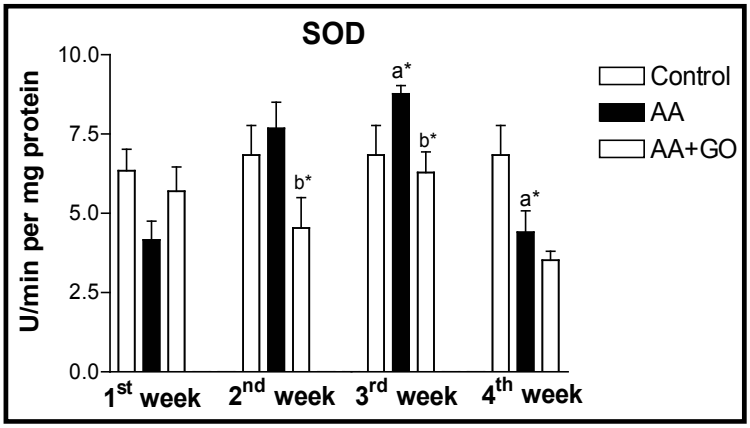


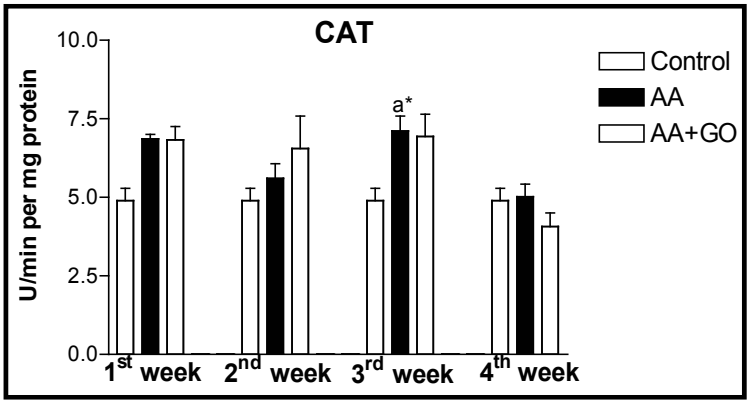
Figure 3 The level of LPO and NO in testicular tissues of control and treated rats at different time intervals of ACR exposure
Letter [a] indicates a significant differences between ACR and control groups (* *P*<0.05 & ** P<0.01)
Letter [b] indicates a significant differences between ACR plus garlic oil and ACR groups (* *P*<0.05 & ** P<0.01)

level. Also, **Figure 4** showed that co-treatment of rats with garlic oil little bed attenuated GSH depletion by ACR. Similar result was obtained by treatment with lipoic acid and garilic [37, 43]. These increases in GSH level with a decrease in LPO might be attributed to the oxidative damage repairing ability of garlic oil.

Superoxide radical may execute several functions: oxidize SH groups, undergo dismutation to form H_2O_2 and singlet oxygen, and react with oxidized iron ions to restore them [44]. In the current study, **Figure 4** showed alteration in the testicular SOD and CAT activities depending on the period of treatments. Also, it showed that co- treatments of rats with garlic oil ameliorated these changes by different levels. These results are in agreement with Abd El-Halim and Mohamed [37] who found that administration of ACR caused a significant reduction in the activity of SOD in testes tissues. And treatment with garilic prior to ACR attenuated the reduction of SOD activity. In addition, Abd El-halim and EL-Adawi [45] found that treatment with the powder of *Curcuma longa L* significantly decreased SOD activity in testes. Finally, Scicchitano *et al*. [46] concluded that nutraceuticals are able to interact with several biochemical pathways in lipid metabolism; hence, they have the potential to overcome the genetic variability of individuals.

From the present results it can be concluded that **e**xposure of rats to ACR caused testicular oxidative stress associated with histopathological changes in seminiferous tubules and reduction in testosterone in serum. Co-treatment of rats with garlic oil ameliorate 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.





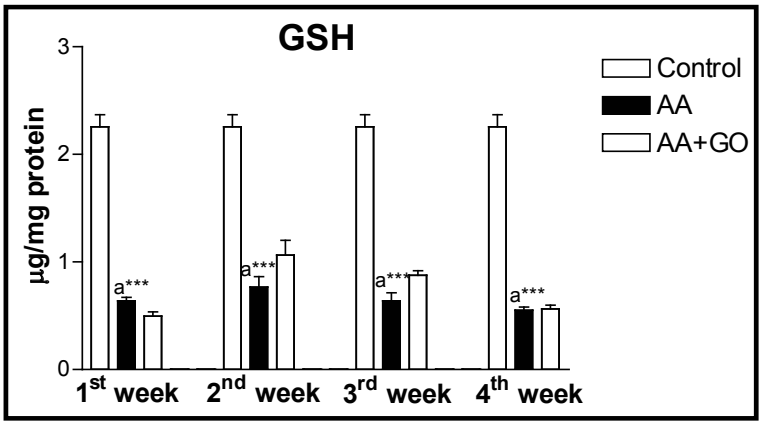


Figure 4 The activity of SOD and CAT and the level of GSH in testicular tissues of control and treated rats at different time intervals of ACR exposure

Letter [a] indicates a significant differences between ACR and control groups (* $P<0.05$ & ** $P<0.01$)

Letter [b] indicates a significant differences between ACR plus garlic oil and ACR groups (* $P<0.05$ & ** $P<0.01$)

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