Original Article Ann Med Res 2022;29(4):397–400

Current issue list available at AnnMedRes
Annals of Medical Research
journal page: www.annalsmedres.org

Effects of acrylamide and crocin on rat lung tissue

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ARTICLE INFO

Keywords:
Lungs
Crocin
Acrylamide
Oxidative stress parameters

Received: Jun 29, 2021
Accepted: Sep 24, 2021
Available Online: Apr 29, 2022

DOI:
10.5455/annalsmedres.2021.06.477

Abstract

Aim: We aimed to determine the effects of acrylamide (AA) and crocin (Cr) on rat lung tissues.

Materials and Methods: Forty Wistar albino rats were divided into control, AA, Cr, and AA + Cr groups. Rats were administered 25 mg/kg AA and 50 mg/kg Cr for 21 days. After 21 days, malondialdehyde (MDA), reduced glutathione (GSH), total antioxidant status (TAS), total oxidant status (TOS), oxidative stress index (OSI), superoxide dismutase (SOD), catalase (CAT) and protein levels were measured in rat lung tissues.

Results: The analysis of the rat lung tissues revealed that oxidant parameter markers (MDA, TOS, OSI) increased and antioxidant parameter markers (GSH, TAS, SOD, CAT) decreased in the AA group when compared to all other groups ($p < 0.05$). A significant increase was determined in antioxidant capacity (GSH, TAS, SOD, CAT) in the Cr-treatment group when compared to all other groups ($p < 0.05$). We found a significant improvement in oxidant-antioxidant parameters in the AA + Cr group when compared to the AA group ($p < 0.05$).

Conclusion: This study was the first of its kind in the literature and revealed that AA administration led to damages in lung tissue. It could be suggested that this was due to an increase in oxidant levels and oxidative stress. Cr exerted a powerful antioxidant effect in lung tissues. Against AA toxicity, we recommend the consumption of Cr to improve antioxidant capacity.

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Introduction

Acrylamide (AA) is a chemical product formed by a reaction between amino acids and sugars. It is generally produced when food with high starch content, such as potatoes, root vegetables, and bread are fired, roasted or baked at high temperatures (above 120 °C) [1, 2]. AA is not an intentional food additive but a natural byproduct of the cooking process and could be produced when any food is cooked above a certain temperature. AA production occurs during a process called the Maillard reaction when food is cooked under high temperatures. The natural water, sugar and amino acid content combines to create the flavor, texture, color and smell of a food item. The same process could also produce AA [3]. AA was classified as a Group 2A Carcinogen by the International Agency for Research on Carcinogens. Occupational Safety and Health Administration and the National Institute of Occupational Safety and Health determined the skin exposure limit as 0.03 mg/m^3 per 8-hour daily shift [4, 5]. In experimental models, exposure to AA led to adrenal gland, thyroid, lung, over and testicle tumors [6-10]. AA could be metabolically activated by cytochrome P-450 to its genotoxic metabolite and this was observed as the critical step in AA carcinogenesis. In reverse, AA and glycinamide could be detoxified by combining with glutathione to form acrylamide and isomeric glycinamide-glutathione compounds, then could be metabolized into mercapturic acid and excreted in urine [11].

The saffron plant is indigenous to Anatolia. It has been cultivated in Anatolia for 3000 years [12]. Crocin (Cr) is an active component of saffron (Crocus sativus L.), which is commonly cultivated and recycled in the world, but mainly in Safranbolu region in Turkey [13-15]. Cr is a natural carotenoid compound found in crocus and gardenia flowers. It is a diester that includes a disaccharide called gentiobiose and a dicarboxylic acid called crocetin. Its color is bright red and forms crystals that liquefy at

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186 °C. It forms an orange solution in water. Cr is the chemical component that is culpable for the color of saffron [16-18]. Saffron and its active ingredients have antitumor, antioxidant, anti-genotoxic, memory and learning enhancing, anti-inflammatory, anticonvulsant, antidepressant, blood pressure regulating, oxygen-enhancing, bronchodilator and protective properties against oxidative stress. Saffron has several applications in various ailments used in traditional medicine [19-25].

These experimental studies aimed to biochemically examine the dose and time-dependent changes in rat lung tissues after AA and Cr administration.

Materials and Methods

Animals

This experimental study was conducted with forty 225-250 g Wistar albino male rats obtained from Inonu University, Faculty of Medicine, Experimental Animal Breeding and Research Center (INUTF-DEHUM). Before the study, experimental animal ethics committee approval (2016/A-59) was obtained. Rats were kept in cages with 21 °C ambient temperature, 55-60% humidity, and 12 hours of light (08:00 to 20:00 hours) and provided with tap water and the cages were cleaned every day during the experiments. Rats were fed ad libitum with standard pellet feed in the study.

Study Design

The Wistar rats (n=40) were allowed randomly to four groups of equal number of rats before the experiment.
Control group: Saline solution was executed.
Acrylamide group (AA): 25 mg/kg AA was executed.
Crocin (Cr) group: 50 mg/kg Cr was executed.
AA + Cr group: 25 mg/kg AA + 50 mg/kg were executed.
Administrations were conducted at the same hour each day in 1 ml/kg/day gavage per rat for 3 weeks.

Preparation of the Tissues for Biochemical Analysis

Lung tissues (-80 °C) were removed from the freezer and weighed before the analysis. Phosphate buffer was added to obtain 10% homogenate and the homogenate was homogenized in ice for 12 minutes at 12000 rpm (IKA, Germany). The tissue homogenates were centrifuged at 5000 rpm and +4 °C for 30 minutes to obtain the supernatant.

Measurement of Malondialdehyde (MDA) Levels

The MDA analysis was conducted with the Uchiyama and Mihara method [26]. The MDA concentration was determined based on the measurements conducted on the supernatant, which was extracted from the n-butanol phase of the pink-colored product produced by the reaction between the MDA in the supernatant with thiobarbituric acid at 95 °C. The measurement was conducted with a spectrophotometer at 535 and 520 nm wavelengths. The results are indicated as nmol/g wet tissue.

Measurement of Reduced Glutathione (GSH) Levels

The GSH levels were determined with the Ellman method [27]. The reaction between the GSH in the analysis tube and 5-dithiobis 2-nitrobenzoic acid produces a yellow-green color. The light intensity of this color was measured with a spectrophotometer at 410 nm wavelength to determine the GSH concentration. The results are presented as nmol/g wet tissue.

Measurement of Superoxide Dismutase (SOD) Levels

The total reduction in nitro blue tetrazolium by the superoxide anion production induced by xanthine and xanthine oxidase provides the SOD activity [28]. The protein content that inhibited 50% of the NBT reduction provides unit SOD activity, and the findings are reported in units per milligram protein.

Measurement of Catalase (CAT) Levels

Aebi method was employed to determine the CAT activity [29]. In the method, constant rate k (dimension: s-1, k) H_2O_2 (10 mM of initial concentration) is determined with the absorbance at 240 nm. CAT activity is reported as K/g protein.

Measurement of Total Oxidant Status (TOS) Levels

ELISA method adjusted to 25 °C was employed to determine the TOS as per the kit instructions. The absorbance of the 500 μL reactive 1 (measurement buffer) and 75 μL serum mixture was measured at 530 nm. After 25 μL reactive 2 (pro-chromogen solution) was added to the mixture, it was incubated for 10 minutes, and absorbance at 530 nm provided the TOS (30). TOS activity is reported as μmol H_2O_2 Equiv/L.

Measurement of Total Antioxidant Status (TAS) Levels

ELISA method adjusted to 25 °C was employed to determine the TAS as per the kit instructions. The absorbance of the 500 μL reactive 1 (measurement buffer) and 75 μL serum mixture was measured at 660 nm. After 25 μL reactive 2 (colored ABTS solution) was added to the mixture, it was incubated for 10 minutes, and absorbance at 660 nm provided the TAS [3]. TAS activity is reported as mmol Trolox Equiv/L.

Oxidative stress index (OSI) Levels

OSI is determined by dividing TOS by TAS. OSI was calculated with the following formula: OSI (arbitrary unit) = TOS (micromole H_2O_2 Eqv/L) / TAS (millimole Trolox Eqv/L) X 10 [30].

Statistical Analysis

To detect an effect size of 0.58 among the groups at 95% confidence level and 80% power, the required minimum sample size per group was calculated as 10.

The normal distribution of the quantitative data was evaluated by Shapiro-Wilks test and data are presented by mean and standard deviation. Homogeneity of the group variances was tested with the Levene test. One-way ANOVA and Tukey HSD post-hoc analysis were employed for the groups with homogeneous variances; Welch test and Games-Howell post-hoc analysis were used for the groups with non-homogenous variances. The significance level was accepted as 0.05.

Table 1. Oxidant-antioxidant some parameters of all groups

Groups	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g protein)	CAT (K/g protein)
C	672.04±26.27 [a]	596.93±20.64 [a]	44.88±2.71 [a]	4.61±0.18 [a]
Cr	345.75±33.52 [b]	917.92±46.9 [b]	66.42±2.93 [b]	8.58±0.15 [b]
AA	961.29±24.03 [c]	338.1±21.56 [c]	24.32±2.58 [c]	1.61±0.23 [c]
Cr + AA	556.09±24.01 [d]	446.48±26.23 [d]	36.49±3.06 [d]	2.92±0.25 [d]

C; Control, Cr; Crocin, AA; Acrylamide, Cr + AA; Crocin + Acrylamide; MDA, malondialdehyde; GSH, reduced glutathione; SOD, superoxide dismutase; CAT, catalase; Data are expressed Mean ± SD ten animals (n=10). gwt; gram wet tissue. Groups with different letters in columns are significantly different from each other ($p < 0.05$).

Table 2. Oxidative stress index parameters of all groups

Groups	TAS	TOS	OSI
	(mmol/L)	(µmol/L)	(AU)
C	1.4±0.05 [a]	22.03±1.37 [a]	157.6±11.97 [a]
Cr	2.91±0.1 [b]	12.14±0.55 [b]	41.6±2.59 [b]
AA	1.03±0.05 [c]	35.58±1.68 [c]	348.3±26.64 [c]
Cr + AA	1.19±0.04 [d]	16.95±1.15 [d]	141.7±9.49 [d]

C; Control, Cr; Crocin, AA; Acrylamide, Cr + AA; Crocin + Acrylamide; TAS, total antioxidant status; TOS, total oxidant status; OSI, Oxidative stress index. Data are expressed Mean ± SD of ten animals (n=10). Groups with different letters in columns are significantly different from each other ($p < 0.05$).

Results

Analysis of the rat lung tissues revealed a significant difference between all groups. In the AA group, there were significant increases in MDA, TOS and OSI levels and significant decreases in GSH, SOD, CAT and TAS levels. In the Cr group, increases in GSH, SOD, CAT and TAS levels and decreases in MDA, TOS and OSI levels were observed. The oxidant - antioxidant parameters improved in the AA + Cr group when compared to the AA group (Tables 1 and 2).

Discussion

The lungs are located in the thoracic cavity where gas exchange occurs between the main vessels and breathed air and blood located next to the heart. The lung tissues are soft, spongy and elastic. The mean lung weight is 1200-1300 grams. The lungs serve to breathe the external air in and allow the oxygen in the air to pass to the capillary blood vessels around the alveoli. The second main task of the lungs is to transfer the carbon dioxide generated by the organs in the venous blood through the alveoli to the external environment. The lungs also function as a metabolic organ. For example, a certain portion of the alcohol, anesthetic agents, etc. in the system are exhaled through the lungs. They also maintain the body pH balance [32]. The studies conducted on experimental animals reported that AA led to lung damage [1]. AA increases the oxidant content and decreases the antioxidant content, leading to oxidative damage.

Hajimohammadi et al. administered AA to rats and investigated its effects on lung tissues. They reported that AA expanded MDA levels and diminished SOD and CAT levels in lung tissues [33]. Ghorbel et al. administered 20 mg/kg/bw AA to rats for 21 days and investigated its effects on lung tissues. They reported that MDA levels increased and GSH, SOD and CAT levels diminished in the AA group when compared to the control group, [34]. Conti et al. administered 0.0875, 0.175, 0.35 and 0.70 mM AA through the drinking water to the rats for 28 days and investigated the effects on lung tissues. They reported that AA led to DNA damage in lung tissue based on the administration dose [35]. Batoryna et al. carry out a single dose 26 µg AA to rats and investigated its effects on lung tissues. They observed that GSH, SOD, and CAT levels diminished in lung tissues after AA administration [36]. In the present study, biochemical findings revealed an expanded in MDA and TOS levels and a diminished in TAS, SOD and CAT levels after AA administration. This present examine findings were parallel with the previous reports.

Marwa et al. developed a bleomycin-induced pulmonary fibrosis model and administered Cr as a protective agent. After 4 weeks, they examined the rat lung tissues. They reported that while bleomycin led to damages in lung tissues, they observed a decrease in expanded MDA levels, diminished SOD, CAT and TAC levels after Cr administration [37]. Wang et al. administered lipopolysaccharide and 50 mg/kg of Cr as a protective to rats. They Reported that Cr application may play a role against lipopolysaccharide toxicity [38]. Yasri et al. developed an allergic asthma model in rats and administered 25 mg/kg Cr as a protective agent. They reported increased SOD, CAT and GSH and decreased MDA levels decreased after Cr administration [39]. In this present examine Cr administration increased TAS, SOD and CAT levels, and decreased MDA and TOS levels. The oxidant - antioxidant parameters reported in the present study are similar to previous study findings.

Conclusion

In the present study, the properties of AA and Cr on lung tissues were studied for the first time in the literature. AA led to tissue damage through causing oxidative stress in rat lung tissues. Cr could play a protective role by increasing the antioxidant capacity against AA toxicity due to its strong antioxidant properties. Further comprehensive studies are required on the topic.

Acknowledgment

The authors thank Prof. Dr. Yusuf Türköz and Prof. Dr. Ahmet Ümit Erdemli for theirs kind helps.

Ethical approval

The decision of the ethics committee of Inonu University Faculty of Medicine numbered 2016/A-59.

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