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Original Article

The role of Crocin in an acrylamide-induced neurotoxicity model in Wistar rats

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Abstract

Aim: The changes in rat brain tissues treated with Crocin (Cr) as a protective agent in an acrylamide (AA) neurotoxicity model were investigated.

Material and Methods: The present with of the experimental animal ethics committee at Inonu University, Faculty of Medicine (2016 / A-59). Forty male rats were randomly divided into 4 groups with study was conducted the approval equal number of rats (10): Control, Cr, AA, Cr + AA Groups. Malondialdehyde (MDA), reduced glutathione (GSH), total antioxidant status (TAS), total oxidant status (TOS), Oxidative stress index (OSI), superoxide dismutase (SOD), catalase (CAT) and protein values were examined in the brain tissues.

Results: MDA, TOS and OSI levels increased in brain tissues of AA administered rats when compared to the other groups, while the GSH, TAS, SOD and CAT levels decreased in the group ($p < 0.05$). GSH, TAS, SOD and CAT levels increased, but MDA, TOS and OSI levels decreased in the AA + Cr administered group when compared to the AA group ($p < 0.05$). It was observed that oral AA administration altered the antioxidant/oxidant balance favoring the oxidants in male rat brain tissues, leading to oxidative stress induced neurotoxicity, while Cr administration reestablished the normal antioxidant/oxidant balance, preventing the oxidative stress induced neurotoxicity via detoxification.

Conclusion: The present study concluded that the administered Cr dose was sufficient to prevent neurotoxicity and we recommend that adequate amounts of Cr should be consumed to prevent AA-induced toxicity and improve antioxidant capacity.

Keywords: Brain; acrylamide; crocin; oxidative stress parameters.

INTRODUCTION

Acrylamide (AA) is commonly used in several industries including printing and textile, and in research laboratories (1,2). Acrylamide is a toxic substance with high chemical activity, and it could be only synthesized chemically and is not present in the nature. AA is formed spontaneously when carbohydrate and protein-rich foods reach temperatures above 120 °C (3). Constant exposure to food-induced AA toxicity leads to health risks for humans. Previous studies determined that AA has neurotoxic, genotoxic and carcinogenic and high toxic effects on animal reproductive systems and it was classified as a neurotoxic and 2A class carcinogenic agent for humans (4,5) AA leads to oxidative stress in living organisms by damaging the oxidant/antioxidant balance (6,7). In general, acrylamide reduces tissue glutathione (GSH) levels and total antioxidant capacity (TAS) and increases lipid peroxidation and total oxidant capacity (TOS) in tissues (8). The resulting tissue damage is caused by the oxidative stress induced by acrylamide exposure (9,10).

Saffron (Crocus sativus L.) is an iridaceous plant indigenous to Greece, Azerbaijan, Austria, Spain, Iran, China, Morocco, Mexico, Libya, Kashmir (India and Pakistan) and Turkey in Iridaceae family. Saffron is the dried red stigma obtained from this plant and saffron is predominantly consumed as a spice and a coloring agent (11). Due to its anticarcinogenic, neuroprotective, hypolipidemic, anti-inflammatory, and antioxidant properties, saffron is also used in alternative medicine (12,13). The active ingredients of saffron include Cr (crocetin glycoside), crocetin and safranal. It also contains flavonoids, amino

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acids, important minerals, vitamins, proteins, sugars, and other chemical substances (14-16). Recently, several research were conducted on neuroprotective effects of saffron and its bioactive component Cr in animal models (17,18).

The present study aimed to determine the impact of the Cr, which was reported to have neuroprotective properties against neurotoxicity induced by AA that is commonly ingested involuntarily, especially due to the prevalence of the fast food culture.

MATERIAL and METHODS

Animals

The present study subjects included 40 male Wistar albino rats, 225-250 grams each, procured from Inonu University, Faculty of Medicine, Experimental Animal Breeding and Research Center (INUTF-DEHUM). Experimental animal ethics committee approval (2016 / A-59) was obtained prior to the study. Drinking water was provided for the rats daily during the experiment and the cages were also cleaned daily. The rats were kept in an environment with 21 °C ambient temperature, 55-60% humidity, and 12 hours of light (08:00 to 20:00 hours) and 12 hours of darkness. Rats were fed ad libitum with standard pellet feed during the experiments.

Study Design

The Wistar rats (n=40) were selected randomly and divided into four equal groups before the experiment.

Control group: Only saline solution was administered.
Acrylamide group (AA): 25 mg/kg AA was administered.
Crocin (Cr) group: 50 mg/kg Cr was administered.
AA + Cr group: 25 mg/kg AA + 50 mg/kg were administered.
All applications were regularly conducted at the same hour everyday with 1 ml/kg/day gavage per rat for 21 days.

Preparation of the Tissues for Biochemical Analysis

Brain tissues that were kept in the freezer (-80oC) were removed and weighed on the day of analysis. Phosphate buffer was added to produce 10% homogenate and the product was homogenized in ice for 12 minutes at 12000 rpm (IKA, Germany). The supernatant was obtained by centrifuging the tissue homogenates at 5000 rpm and +4 oC for 30 minutes.

Measurement of Malondialdehyde (MDA) Levels

The MDA analysis was conducted with the method proposed by Uchiyama and Mihara (19). The MDA concentration was determined with supernatant measurement, which was extracted from the n-butanol phase of the pink-colored product revealed due to the presence of MDA in the supernatant that reacted with thiobarbituric acid at 95 °C, using a spectrophotometer at 535 and 520 nm wavelengths. The results are indicated in nmol/g wet tissue.

Measurement of Reduced Glutathione (GSH) Levels

The GSH levels were determined with the method reported by Ellman (20). The GSH present in the analysis tube reacted with 5'-dithiobis 2-nitrobenzoic acid, resulting in a yellow-greenish color. To determine the GSH concentration, light intensity of this color was measured with a spectrophotometer at 410 nm wavelength. The results are presented as nmol/g wet tissue.

Measurement of Superoxide Dismutase (SOD) Levels

SOD activity was identified with the total reduction in nitro blue tetrazolium by superoxide anion production induced by xanthine and xanthine oxidase (21). Unit SOD activity was measured by the quantity of protein that inhibited NBT reduction by 50%, and the findings are reported in units per milligram protein. SOD activity is indicated as U/g protein.

Measurement of Catalase (CAT) Levels

CAT activity was determined with the method proposed by Aebi (22). This method is used to determine the constant rate k (dimension: s-1, k) H2O2 (10 mM of initial concentration) based on the absorbance at 240 nm. CAT activity is reported as K/g protein.

Measurement of Total Oxidant Status (TOS) Levels

TOS levels were determined with the ELISA method adjusted to 25oC as indicated in the kit instructions. 500 µL reactive 1 (measurement buffer) and 75 µL serum were mixed, and the absorbance was measured at 530 nm wavelength. 25 µL reactive 2 (pro-chromogen solution) was added to the mixture, the product was incubated for 10 minutes, and TOS levels were measured with the absorbance at 530 nm (23). TOS activity is reported as µmol H2O2 Equiv/L.

Measurement of Total Antioxidant Status (TAS) Levels

TAS levels were determined with the ELISA method adjusted to 25oC as indicated in the kit instructions. 500 µL reactive 1 (measurement buffer) and 75 µL serum were mixed, and the absorbance was measured at 660 nm. 75 µL reactive 2 (colored ABTS solution) was added to the mixture, the product was incubated for 10 minutes, and TAS levels were measured with the absorbance at 660 nm (24). TAS activity is reported as mmol Trolox Equiv/L.

Oxidative stress index (OSI) Levels

OSI is the ratio of TOS to TAS value. OSI was calculated with the following formula: OSI (arbitrary unit) = TOS (micromole H2O2 Eqv/L) / TAS (millimole Trolox Eqv/L) X 10.

Statistical Analysis

Normal distribution of the data was analyzed with the Shapiro-Wilk test and the study data were summarized with mean and standard deviation values. Homogeneity of the variances between the groups was tested by the Levene test. When the group variances were homogeneous, one way ANOVA and Tukey HSD post-hoc analysis were used; otherwise Welch test and Games-Howell post-hoc analysis were used for non-homogenous variances. In all analyses, significance level was accepted as 0.05.

RESULTS

Biochemical analysis of the brain tissue demonstrated that there was a difference between the control group and

all other groups. In the AA administered group, MDA, TOS and OSI levels increased, while GSH, SOD, CAT and TAS levels decreased when compared to all other groups. In the Cr administered group, GSH, SOD, CAT and TAS levels increased and MDA and TOS levels decreased when compared to all other groups. In the AA + Cr administered group, MDA, TOS and OSI levels decreased and GSH, SOD, CAT and TAS levels increased when compared to AA group (Tables 1 and 2).

Table 1. Oxidant-antioxidant some parametrs of all groups

Groups	MDA (nmol/gwt)	GSH (nmol/gwt)	SOD (U/g protein)	CAT (K/g protein)
C	1072 ± 116 [a]	678 ± 38 [a]	63 ± 3.6 [a]	1.5 ± 0.1 [a]
Cr	914 ± 178 [b]	907 ± 43 [b]	84 ± 3.1 [b]	4.5 ± 0.2 [b]
AA	1602 ± 182 [c]	535 ± 36 [c]	45 ± 3 [c]	0.6 ± 0.08 [c]
Cr + AA	1146 ± 75 [d]	727 ± 44 [d]	57 ± 3 [d]	1.3 ± 0.13 [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 (mmol/L)	TOS (µmol/L)	OSI (AU)
C	0.6 ± 0.02 [a]	12.4 ± 0.08 [a]	206 ± 6 [a]
Cr	1.3 ± 0.03 [b]	6.2 ± 0.2 [b]	47 ± 7 [b]
AA	0.37 ± 0.05 [c]	22 ± 2.2 [c]	594 ± 9 [c]
Cr + AA	0.8 ± 0.04 [d]	15.4 ± 1.3 [d]	192 ± 8 [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 ($p < 0.05$)

DISCUSSION

The aim of the current experimental study was to research protective effects of Cr on oxidative stress induced by AA. AA is a water-soluble substance with high toxicity that could permeate the tissues easily after ingestion. AA is produced when nutrients with high protein and carbohydrate content are cooked above 120 °C. Long-term ingestion of this substance through contaminated water and nutrients leads to exposure of the human body to AA. AA has toxic and carcinogenic properties (25). The destruction of the oxidant/antioxidant balance in favor of oxidants leads to oxidative damage. Living organisms have antioxidant defense systems to control free radical formation (26). However, these systems are not efficient in removal of the adverse effects of free radicals in all cases, which results in oxidative damage. In these cases, antioxidant agents are required to remedy the oxidative damage (27).

The body includes endogenous antioxidant enzymes that form an active defense system, which functions to neutralize the induced by free radicals (28). The endogenous defense system includes SOD and CAT enzymes that work against oxidative stress and have an important role against the adverse physical effects of lipid and hydrogen peroxidation (29). GSH is as a non-enzymatic antioxidant that plays an important role in balancing the physical antioxidant defense mechanism (30). The present study aimed to determine the antioxidant properties of Cr through the identification of antioxidant enzyme (SOD and CAT) activities in the brain. The destructive AA induced neurotoxicity process is a product of lipid peroxidation. The present study demonstrated that oral AA administration induced free radical formation led to cellular damage in rat brains.

Erdemli et al. studied the effects of AA and N-acetylcysteine (NAC) on fetal brain tissues in a gestation model. As a result of AA administration during pregnancy, it was reported that MDA levels increased, CAT levels decreased and AA administration led to neurotoxicity in fetal brain tissues (31). In another study conducted with the gestation model, Erdemli et al. administered vitamin E as a protective agent against AA-induced neurotoxicity. They examined the fetal brain tissues after 20 days. It was reported that MDA, TOS levels increased and GSH and TAS levels decreased in the AA administered group when compared to all other groups (32). In an AA-induced neurotoxicity model (20 mg / kg), Goudarzi et al. administered AA and ellagic acid (EA) as a protective agent for 30 days. At the end of the 30 day period, they examined the brain tissues of the rats. They reported that AA administration led to an increase in MDA, GSH, and SOD levels and a significant decrease in CAT levels when compared to all other groups (33). Tabeshpour et al. administered 50 mg/kg AA and 2.5, 5 and 10 mg/kg thymoquinone (TQ) as a protective for 11 days. On the 12th day, they examined the brain tissues of the rats. They found that MDA levels increased and GSH levels significantly decreased in the AA administered group when compared to all other groups (34).

Mehri et al. administered 50 mg / kg AA and 12.5, 25, 50 mg / kg Cr for 11 days. At the end of this period, they examined the brain tissues of the rats. It was observed that MDA and GSH levels increased in the AA administered group, while MDA levels decreased and GSH levels increased in a dose-dependent manner in the Cr administered group (35). In a rotenone (ROT) induced neurotoxicity model, Rao et al. administered 25 mg / kg Cr as a protective for 7 days. On the 8th day, they examined the changes in rat brain tissues. They reported that the MDA levels that increased with ROT administration, and GSH, SOD and CAT levels that decreased with ROT administration approached to

control group levels and exhibited improvement with Cr administration (36). In a methamphetamine (METH) induced neurotoxicity model, Shafahi et al. administered 30, 60, 90 mg / kg Cr as a protective agent. They reported that Cr administration increased SOD levels and decreased MDA levels in rat brain tissues (37).

The present study findings on oxidant-antioxidant parameters and oxidative stress index were consistent with the reports of previous studies. It was demonstrated that AA led to oxidative stress damage in male rat brain tissues and Cr exhibited strong antioxidant properties that could inhibit AA-induced oxidative stress.

CONCLUSION

It was determined that oral AA ingestion caused neurotoxicity induced by oxidative stress in male rat brain tissues through the destruction of the antioxidant / oxidant balance in favor of oxidants. Cr administration acted as a protective against oxidative stress induced neurotoxicity by balancing the antioxidant and oxidant levels via detoxification. In the present study, it was determined that the administered dose was adequate for neurotoxicity prevention and consumption of suitable Cr doses was recommended for protection against AA-induced toxicity and improves antioxidant capacity. Further studies are required to discover all mechanisms involved in AA induced neurotoxicity in adult brain tissues.

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