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Research Paper

A NEUROPROTECTIVE ROLE FOR FERULIC ACID AGAINST ACRYLAMIDE -INDUCED NEUROTOXICITY IN RATS

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Abstract

Acrylamide (ACR) is a neurotoxic, genotoxic, and carcinogenic compound. Ferulic acid, a phyto-constituent (FA), which has antioxidant and anti-inflammatory activities. The objective of the current study is to investigate the neuroprotective role of FA treatment on acrylamide ACR-induced oxidative damage of lipid, protein and DNA in rat brain. Forty adult male albino rats were divided into four groups as follows: control, FA (25 mg/kg), ACR (20 mg/kg) and FA+ACR groups. ACR and FA were administered orally by gavage for 30 days. The results showed that administration of ACR caused a locomotor abnormalities and muscular distension. In addition, individual treatment of ACR and FA induced significant decrease ($P<0.05$) in brain acetylcholine esterase (AChE) activity. Moreover, ACR significantly increased the levels of tumor necrosis factor-α (TNF-α), nitric oxide (NO), 8-hydroxydeoxyguanosine (8-OHdG), malondialdehyde (MDA) and protein carbonyls (PC) levels, as indicators of oxidative damage of DNA, lipid and protein, respectively, in rat brain. In addition, ACR treated rats exhibited decreased glutathione (GSH) content and also inhibited enzymatic activity of glutathione-S-transferase (GST) and glutathione peroxidase (GPX) activity in the brain, indicating the occurrence of oxidative stress. Combined treatment resulted in ameliorative effect against ACR toxicity, where it minimized the oxidative damage, and remarkably antagonized the decreasing effect of ACR on both monoamines and free amino acid in rat brain tissues. Despite that both ACR and FA share an inhibitory effect on AChE activity, ACR's effect represent neurodegenerative effect, whereas FA might represent an activation or modulation for cholinergic neurotransmission,. Thus, the study indicated that FA provided a neuroprotective effect against ACR-induce neurotoxicity in rat.

Key words: acrylamide, ferulic acid, rats, oxidative stress, Acetylcolinesterase, monoamines, amino acids, brain.

INTRODUCTION

Acrylamide (ACR) has been classified as a neurotoxic, genotoxic and carcinogenic substance [1-3]. Maillard browning is proposed as the most probable mechanism within several hypotheses for the development of acrylamide formation in cooked foodstuff from the chemical reaction between reducing sugars and free amino acids especially asparagines [4-5]. Acrylamide caused oxidative stress like affection of lipid peroxidation as well as reduction of glutathione (GSH) and

catalase enzyme [6]. Consistently, high doses of acryalmide induced oxidative stress with the loss of free radical scavenging activity in discrete regions of heart [7]. ACR induced a significant DNA damage in the lymphocyte and liver cells and the micronucleus formation in bone marrow cell were significantly alleviated [8]. Acrylamide is metabolized by hepatic CYP 2E1 producing glycidamide, which is reactive toward protein and nucleic acid nucleophiles [9,10]. Despite that acrylamide is known to be a neurotoxic, genotoxic, and carcinogenic compound, the same dose of its metabolite- glycidamide- had more toxic effects and damage effects to the mice compared to that of acryalmide [11,12].

Ferulic acid [(E)-3-(4-hydroxy-3-methoxy-phenyl) prop-2-enoic acid)] is a common polyphenolic compound found abundantly in vegetables particularly artichokes and eggplants [13]. Protein oxidation and lipid peroxidation were reduced by FA in hippocampal neuronal cells [14] and in female rat livers [15]. Moreover, the expressions of endothelial and inducible nitric oxide synthases (NOS) in mouse hippocampus [16] and in rat cortical neurons [17] were inhibited after FA administration. Ferulic acid had protective effects against various diseases such as cancer, diabetes, and neurodegenerative diseases [17,18]. Ferulic acid plays a neuroprotective role against transient focal cerebral ischemia through its anti-oxidant and anti-apoptotic effects [19]. Ferulic acid reduces focal cerebral ischemic injury by modulating the expression of apoptosis-related proteins and regulating inflammatory reactions [19,20]. Moreover, ferulic acid protects cortical neuronal cells against glutamate toxicity and modulates the phosphatidylinositol 3-kinase (PI3K) and extracellular signal regulated kinase (ERK) pathways [21].

The aim of present study is to investigate the neuroprotective effect of FA on acrylamide-induced toxicity in rat.

MATERIALS AND METHODS

Chemicals

Acrylamide, glutathione (GSH), 5,5´-dithiobis 2-nitrobenzoic acid (DTNB), thiobarbituric acid (TBA) 2,4-dinitrophenyl hydrazine (DNPH), guanidine hydrochloride and Griess reagent were purchased from Merk-Schuchardt Chemical Company (Hohenbrunn, Germany), with purity of 99%. Ferulic acid was obtained from Sigma Chemical Company, USA. All other chemicals were of analytical grades.

Grouping of animals

Rats were divided into four equal groups (n=10) and treatment was given as follows:
Group 1- control.
Group 2- acrylamide (20 mg/kg, p.o).
Group 3- ferulic acid alone (25 mg/kg, p.o).
Group 4- ferulic acid (25 mg/kg p.o)+acrylamide (20 mg/kg, p.o)
Acrylamide and ferulic acid were given by oral gavage administrations for 30 days.

The visual observation of gait problems

Behavioral testing was performed on the 30th day. After completion of treatment, the rats were placed in a clear plexiglass box and were observed for 3 min. Following observation, a gait score was assigned from a normal, unaffected gait; a slightly affected gait (foot splay, slight hind limb weakness and spread); a moderately affected gait (foot splay, moderate hind limb weakness, moderate limb spread during ambulation) and a severely affected gait (foot splay, severe hind limb weakness, dragging hind limbs, inability to rear) [22].

After completion of visual observation, rats were sacrificed by cervical dislocation to obtain blood and brains. Blood samples were collected and kept without anticoagulant at room temperature for 1h, then centrifuged at 3000 rpm/30min and the separated serum was used for estimation of AChE activity, 8-OHdG and MDA contents. The brains were excise from the skull.

Preparation of brain homogenates

Brains were washed with cold saline (0.9 %). Brains were divided into two halves. One half of brains tissues under investigation were homogenized in ice-cold 1.15% KCl–0.01 M sodium,

potassium phosphate buffer pH 7.4 with a Potter-Elvehjem glass homogenizer to prepare 10 % w/v homogenate. The homogenates were centrifuged at 10,000 rpm for 20 min at 4°C (Sigma-3K30, Germany). The resultant supernatant was used for different enzyme assays. The other halves were homogenized into 70% methanol to prepare 10 % w/v homogenate. This homogenate was used to determine monoamines and free amino acids.

Determination of biochemical parameters:

Nitric oxide (NO) level was as total nitrate using the colorimetric method of Montgomery and Dymock [23]. protein carbonyls (PC) Levels were determined according to method of Levine *et al*. [24] modified by Adams *et al*. [25]. Determination of glutathione-S-Transferases (GST) was carried out according to Habig *et al*. [26]. Glutathione Peroxidase (GPX) assay was determined according to Rotruck *et al*. [27]. Malondialdehyde levels, as a marker of lipid peroxidation, was determined according to the thiobarbituric acid reaction described by Ohkawa *et al*. [28]. Acetylcholinesterase (AChE) Activity was determined according to a Ellmann *et al*. [29]. Reduced glutathione level was assayed in tissue homogenates according to the method of Ellman [30]. Protein content was determined according to method of Lowry *et al*. [31]. Levels of TNF-α and 8-OHdG were quantified using ELISA kits according to the manufacturer's instructions and guidelines. Brain Monoamines was determined by HPLC method [32] and free amino acids were determined by using HPLC method [33].

Statistical analysis

The values were expressed as the mean±SE for the 10 rats in each group. Differences between groups were assessed by one way analysis of variance (ANOVA) using the statistical package for social sciences (SPSS) software package for Windows (version 13.0). Post hoc testing was performed for intergroup comparisons using the least significant difference (LSD) test. A value corresponding to $P<0.05$ was considered statistically significant.

RESULS

Behavioral testing by visual observation:

Acryalmide treated animals exhibited moderate gait problems in comparison to normal control. FA treated animals did not differ from control group. Combined treated animals exhibited slight gait problems

Neurochemical Study:

The activity of AChE activity significantly decreased in the ACR-treated group when compared with the control group ($P<0.05$). FA treatment decreased AChE activity significantly ($P<0.05$) compared with ACR-treated group (Table 1). In the ACR treated group, 8-OHdG and MDA levels significantly increased in the rat serum. In the ferulic acid treated ACR group, these parameters were reversed significantly ($P<0.05$, Table 1).

In the ACR treated group, NO and TNF-α levels significantly increased while, AChE activity significantly decreased in rat brain, compared to control group, FA markedly ($P<0.05$) improved these parameters. In spite of the inhibitory effects of both FA and ACR, combined treatment moderately enhance the enzyme activity compared to ACR treated rats (Table 2).

As an indicator of oxidative damage lipid, protein and DNA, MDA, PC and 8-OHdG levels in rat brain cells were significantly increased in the ACR group and this effect was ameliorated significantly by FA treatment ($P<0.05$, Table 2). In addition, the level of nitric oxide and TNFα a significantly increased in ACR treated rats, combined treatment attenuated ACR effect (Table 2).

Acrylamide treated rats exhibited reduced glutathione levels and decreased catalytic activity of both GST and GPX enzymes, while FA co-treatment significantly ($P<0.05$) reduced this effect (Table 3).

Results depicted in table (4) showed that ACR treatment significantly decreased free amino acids in brains of treated animals. Whereas combined treatment with ferulic acid remarkably minimized ACR decreasing effects on brain free amino acids.

ACR treatment significantly decreased brain monoamines. On the other hand, combined treatment with ferulic acid remarkably minimized ACR decreasing effects on brain monoamines (Table 5).

Table 1 Effects of ferulic acid (FA, 25 mg/kg) on AChE activity and 8-OHdG and MDA levels of rat serum treated with acryalmide (ACR, 20mg/kg,p.o).

Parameters	Control	ACR	FA	FA+ACR
AChE (U/l)	552.64±2.62	263.31±1.81[a]	354.79±2.72[a]	320.09±7.21[ab]
8-OHdG (ng/ml)	1.19±0.09	5.97±0.15[a]	1.07±0.13[b]	2.64±0.14[ab]
MDA (nmol/ml)	5.55±0.05	13.59±0.27[a]	5.30±0.09[a]	7.66±0.48[ab]

Data are expressed as Mean ± S.E. for 6-rats/group
a significant from control group with one way ANOVA at P < 0.05.
b significant from acrylamide group with one way ANOVA at P < 0.05.

Table 2 Effect of ferulic acid (FA,25 mg/kg) on MDA, PC, 8-OHdG, NO and TNF-α levels and AChE activity of rat brain exposed to acryalmide (ACR, 20mg/kg,p.o).

Parameters	Control	ACR	FA	FA+ACR
MDA (nmol/mg protein)	0.37±0.02	2.34±0.15[a]	0.35±0.02[b]	3.38±.08[ab]
PC (nmol /ml)	24.39±0.71	43.53±0.91[a]	22.12± 1.08[b]	23.6± 0.86[b]
8-OHdG (ng/mg protein)	3.81±0.11	7.63±0.29[a]	3.66±0.17[b]	5.31±0.18[b]
NO (µmol/mg protein)	7.81 ± 0.24	17.49± 0.38[a]	6.73 ± 0.32[b]	8.77 ± 0.39[b]
TNF-α (pg/mg protein)	12.54± 0.29	34.22±0.88[a]	10.54±0.23[b]	18. 17± 0.61[ab]
AChE (nmol/min/mg protein)	23.20±0.65[a]	9.76±0.47[a]	14.43±0.80[ab]	13.21±0.58[ab]

Data are expressed as Mean ± S.E. for 6-rats/group
a significant from control group with one way ANOVA at P < 0.05.
b significant from Acrylamide group with one way ANOVA at P < 0.05.

Table 3 Effect of ferulic acid (FA, 25 mg/kg) on GST, GPX activities and GSH content of rat brain exposed to acryalmide (ACR, 20mg/kg,p.o).

Parameters	Control	ACR	FA	FA+ACR
GST (µmol/ min/mg protein)	131.53±2.63	74.62±4.92[a]	129.51±1.69[b]	108.56±2.65[ab]
GPX (µmol/min/mg protein)	20.43±1.02	9.75±0.87[a]	19.94±1.1[b]	13.89±0.77[ab]
GSH (µg/mg protein)	5.39± 0.14	2.68± 0.16[a]	5.65± 0.07[b]	4.47± 0.21[ab]

Data are expressed as Mean ± S.E. for 6-rats/group
a significant from control group with one way ANOVA at P < 0.05.
b significant from Acrylamide group with one way ANOVA at P < 0.05

Table 4 Effect of Ferulic acid (FA, 25 mg/kg, p.o) on levels of free amino acids in rat brain exposed to acryalmide (ACR, 20mg/kg,p.o).

Parameters	Aspartic acid	Glutamic acid	Glycine	Taurine	Histamine	GABA
C	1.663 ± 0.081	2.297 ± 0.137	0.329 ± 0.01	0.231 ± 0.007	0.771 ± 0.057	0.636 ± 0.021
ACR	3.179 ± 0.117[a]	3.874 ± 0.212[a]	0.156 ± 0.012[a]	0.129 ± 0.008[a]	0.421 ± 0.034[a]	0.376 ± 0.028[a]
FA	2.769 ± 0.198[ab]	3.719 ± 0.188[ab]	0.163 ± 0.006[a]	0.105 ± 0.004[a]	0.481 ± 0.036[a]	0.441 ± 0.013[ab]
FA+ACR	2.074 ± 0.079[ab]	2.867 ± 0.052 [ab]	0.22 ± 0.011[a b]	0.211 ± 0.012[b]	0.791 ± 0.061[b]	0.515 ± 0.017[ab]

Data are expressed as Mean ± S.E. for 6-rats/group
a significant from control group with one way ANOVA at P < 0.05.
b significant from Acrylamide group with one way ANOVA at P < 0.05.

Table 5 Effect of Ferulic acid (FA, 25 mg/kg) on Levels of Dopamine (DA). Nor - epinephrine (NE) and Serotonin (5-HT) content of rat brain tissues exposed to acryalmide (ACR, 20mg/kg,p.o).

Parameters	NE	DA	5HT
C	0.657 ± 0.011	1.812 ± 0.085	0.694 ± 0.022
ACR	0.397 ± 0.026[a]	1.02 ± 0.037[a]	0.462 ± 0.014[a]
FA	0.513 ± 0.029[ab]	1.327 ± 0.06[ab]	0.521 ± 0.012[ab]
FA+ACR	0.599 ± 0.027[b]	1.432 ± 0.106[ab]	0.595 ± 0.025[ab]

Data are expressed as Mean ± S.E. for 6-rats/group
a significant from control group with one way ANOVA at P < 0.05.
b significant from Acrylamide group with one way ANOVA at P < 0.05.

DISCUSSION

The present study indicated declined activity of brain AChE in ACR- treated rats. This may cause persistent accumulation of acetylcholine in the synaptic clefts, resulting in an acute cholinergic syndrome via continuous stimulation of cholinergic receptors. In accordance, several studies indicated that ACR induced significant decrease in brain AChE activity in both rat and mice [34,35] On the other hand, Pennisi *et al*. [36] showed that ACR enhanced activity of AChE in peripheral nerves. One of the most important mechanisms of ACR-induced cholinergic dysfunction is related to the ACR reaction with cysteine residues in the presynaptic membranes and inhibition of the neurotransmitter release into the synaptic cleft [37,38]. This suits well the declined AChE activity, which is directly correlated with acetylcholine availability through its release. However, there are other mechanisms influencing AChE activity. One of them may be related to direct binding of ACR and/or its metabolite glycidamide with active –SH sites of the enzyme. The second one may be related to oxidation of the cysteine in the enzyme by free radicals. Both mechanisms participate in AChE inhibition by pesticides [39]. In the present study, the appearance of gait problems in ACR- treated rats might support the involvement of the cholinergic dysfunction in ACR induced neuromuscular deficit. This does not rule out the involvement of other neurotransmission in ACR effects.

The observation that ACR induced significant decrease in the levels of DA, NE and 5-HT in treated rats might indicate that ACR induced common inhibition of monoaminergic neurotransmission. This might be interpreted that ACR might inhibit monoamines synthesis and /or activate their degradation. In accordance, a previous study indicated that ACR increased the catalytic activity of monoamine oxidase (MAO) in rat brain [34]. This might support that active

degradation might cause the decreased brain monoamine levels in ACR treated rat. This might plat a role in the gait problems in ACR treated rats.
In addition, ACR treated rats exhibited decreased levels of free amino acids, which might indicate a disturbing effect of ACR on the levels of both brain excitatory and inhibitory amino acids. In addition, previous studies indicated that certain amino acids, including glycine, histidine, cysteine, glutamine and tryptophan, exhibit anti-inflammatory effects and histidine and glutamine suppress NF-kB activation [40-42]. This might explain the elevated level of TNFα in the present work.

The present study showed that ACR significantly increased levels of MDA, PC and NO; and decreased GSH level and inhibited catalytic activities of SOD and glutathione peroxidase in rat brain. This might indicate that ACR might induce oxidative stress leading to lipid and protein peroxidation. Moreover, the increased level of cytosolic 6 deoxy-8 hydroxyguanosine- a marker of DNA damage and increased the level of TNF-α in brains of ACR treated rats might indicate the occurrence of inflammation and cell apoptosis. Consistently, a previous study showed that ACR and its metabolite glycidamide, enhance the production of reactive oxygen species (ROS) [43]. In addition, a special role is attributed to the SH-containing compounds. Reduced glutathione, the predominant non-protein sulfhydryl in cells, has a dual role in tissues exposed to ACR. The first, GSH conjugates with ACR and/or glycidamide. Second, GSH and other –SH containing compounds take part in the neutralization of free radicals [44]. The conjugation reaction with thiols reduces ACR and glycidamide toxicity. On the other hand, conjugation leads to the depletion of the GSH and other thiol reserves that has negative influence on the redox balance [44]. The oxidation of unsaturated fatty acids produces different compounds. MDA belongs to the secondary products and is used as a convenient marker for lipid peroxidation [45]. We have indicated an increased concentration of MDA and PC in brain of ACR treated rats. Noteworthy, lipid and protein peroxidation play a significant role in neural system disorders including neurodegeneration.. An increased MDA concentration was found in neurofibrillary tangles of Alzheimer's disease brains [46]. Similar data on MDA occurrence after exposure to ACR were presented by Zhu *et al*. [47]. This means that ACR has significant influence on redox balance in brain. These observations suggest that intoxication with ACR may not only affect on-going brain functions, by AChE inhibition and altering the monoaminergic neurotransmission, but may also participate in etiology of neurodegeneration. Accordingly, several studies indicated that oxidative stress is a key mechanism in many ACR induced cell injuries and neurodegenerative diseases [48-50]. In addition, the increased levels of the pro-inflammatory cytokine TNF-α and oxidative DNA damage product, 8-OHdG In ACR treated rats might support this interpretation.

The present findings showed that ferulic acid treatment remarkably reversed the harmful effects of ACR on most parameters except for AChE. Ferulic acid pretreatment restored the normal redox status and reset the normal levels of both brain monoamines and amino acids in ACR treated rats. In accordance, a recent study indicated that ferulic acid reverses the cognitive dysfunction caused by amyloid β peptide 1-40 through anti-oxidant activity and cholinergic activation in rats by activating central muscarinic and nicotinic receptors and anti-oxidant enzymes [51]. FA restored the normal activates of superoxide dismutase, catalase, glutathione peroxidase against the decreasing effect of nicotine [52]**.** In addition, ferulic acid showed anti-inflammatory and antioxidant potentials against vincristine-induced painful neuropathy in rats [53]. Consistently, Khanduja *et al*. [54] showed that FA reduced hydrogen peroxide-induced lipid peroxidation in peripheral blood mononuclear cells. Moreover, a previous study showed that ferulic acid amliorated learning and memory deficits, which might be due to antioxidation, the improvement of cholinergic system in brain, or the inhibitory of nerve injury by excitatory amino acids [55]. In accordance, a previous study indicated that ferulic acid plays a neuroprotective role against transient focal cerebral ischemia through its anti-oxidant and anti-apoptotic effect [56].

In conclusion, the present study might suggest that ACR, induce its detrimental neurotoxic effect on cholinergic neurotransmission, monoaminergic system and free amino acids in brain directly ACR by modulating the activities of the enzymes and receptors in the respected neurotransmission and through disturbing the redox status and initiating the inflammatory process

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