Effect of Acrylamide on neurotransmitters and acetyl-cholinestrase activity in the brain of rats: Therapeutic effect of ferulic acid and selenium nanoparticles

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Received 25 May 2017
Accepted 29 June 2017

ABSTRACT

Objectives: In the present study, the effect of selenium nanoparticles (SeNPs) and ferulic acid (FA) on acrylamide (AC) induced neurotoxicity in rats was investigated.

Methods: Sixty adult male rats were divided equally into six groups and orally treated as follows: Group I (control): 1ml per day physiological saline; group II (SeNPs): 0.5 mg/kg body weight per day SeNPs for 21 days; group III (FA): 100 mg/kg body weight per day FA for 21 days; group IV (AC): 50 mg/kg body weight per day acrylamide for 21 days; group V (AC+SeNPs): AC was administrated as group IV then SeNPs was administrated like group II. Group VI (AC+ FA): AC was administrated as group IV then FA was given as group III.

Results: AC significantly ($P<0.05$) decreased the level of Epinephrine (EPI), norepinephrine (NE) and Serotonin (SER) while, it significantly ($P<0.05$) increased the level of Acetylcholinestrase (AChE) in the brain tissue compared to control group. Treatment with SeNPs significantly ($P<0.05$) increased the level of EPI, NE, and SER in the brain tissue, while treatment with FA significantly ($P<0.05$) increased the level of NE and SER and induced non-significant changes in EPI level. The treatment with FA or SeNPs significantly decreased the level of AChE in the brain tissue compared to the AC group.

Conclusion: It is concluded from the present study that both FA and SeNPs has a potent therapeutic effects against AC induced neurotoxicity in experimental rats.

KEY WORDS: Acrylamide neurotoxicity
Selenium nanoparticles
Ferulic acid

Introduction

Acrylamide (AC) is a neurotoxic in humans and experimental animals that produces skeletal muscle weakness and ataxia that is related to damage to distal axon and nerve terminal regions. AC has extensive use in many fields in industrial manufacturing and has been found in relatively high concentrations in a variety of cooked and heat processed foods frequently consumed by humans. Therefore, the increasing and continuous exposure of humans to AC may lead to nerve damage [1]. The mechanisms of AC neurotoxicity are still unclear, but recent studies have shown that AC neurotoxicity is associated with the enhancement of lipid peroxidation and the reduction of antioxidative capacity in nerve tissue which is mostly caused by a primary depletion of reduced glutathione (GSH) [2,3]. In several cell types, there are acrylamide induced cytotoxic effects via elevation in various types of oxidative markers such as reactive oxygen species (ROS), 3-nitrotyrosine and activation of Cox2 and nitric oxide synthase (NOS) [4,5]. There are two major ways for AC metabolism: direct conjugation with GSH or the formation of glycidamide through oxygenation via cytochrome P450 (CYP2E1). Glycidamide undergoes further biotransformation by hydrolysis to glyceramide or GSH conjugation, resulting in the formation of two mercapturic acid products excreted in the urine [6].

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Glycidamide exerts toxic and mutagenic effects, which reacts with DNA so is considered to be the cancer-initiating agent in acrylamide exposure. Ferulic acid (FA) is a phytochemical commonly found in fruits and vegetables such as tomatoes, sweet corn and rice bran. It exhibits a wide range of therapeutic effects against various diseases like cancer, diabetes [7], cardiovascular and neurodegenerative [8]. FA is considered as a potent antioxidant and this is due to its phenolic nucleus and an extended side chain conjugation, it readily forms a resonance stabilized phenoxy radical [9]. FA also has anticarcinogenic, hepatoprotective, anti-

inflammatory, anti mutagenic and neuroprotective properties [10]. FA can be absorbed by small intestine and excreted in urine, where therapeutic efficacy is dependent on physiological concentration and pharmacokinetic properties, which include absorption, distribution, metabolism and excretion of metabolites [11].

FA is reported as a potent scavenger of ROS and reactive nitrogen species (RNS) generated in the brain so it prevents the DNA and RNA oxidation, lipid peroxidation and neuronal dysfunction or death [12]. FA can have a favorable effect on Alzheimer's disease and Parkinson's disease due to its anti-inflammatory and antioxidant properties. FA also inhibits the formation of β-amyloid fibrils and destabilization of existing fibrils [11]. Selenium (Se) is an essential element for many organisms, including humans, and plays a role in a variety of physiological responses. The selenium used by the human body is gained through various forms found in food and water including selenite, selenate and selenomethionine. The Se content in foods varies considerably. Fruits and vegetables contain trace amount of Se, while cereal, legumes and meat are rich sources of Se in the form of selenomethionine [13]. A main biological importance of Se in an organism is associated with its occurrence in the active centers of numerous enzymatic complexes and in proteins, which are crucial for metabolism. Se is an important component of gluthatione peroxidase, which plays the main protective role against oxidizing activity of hydrogen peroxide and organic peroxides on cell membrane lipids. Selenium, as the component of other enzymes guaranteeing the oxidative and reductive balance and that of b and c cytochromes, takes part in metabolic processes at the level of a cell. Se also has key functions in the proper functions of the immune system and anticarcinogenetic effects [14, 15]. Se plays an important role in the protection from Oxidative stress and ROS, which are strongly implicated in a number of neuronal and neuromuscular disorders, including stroke and cerebrovascular disease, Alzheimer's disease, Parkinson's disease [16]. Nanotechnology holds promise for medication and nutrition because materials and the nanometer dimension exhibit new properties different from those of both isolated atom and bulk material. SeNPs is a newl Se species with novel biological activities and low toxicity compared with other Se compounds like sodium selenite, selenomethinine and methylselenocysteine [17-18].

Materials and Methods

Chemicals

Acrylamide dry crystals (C_3H_5NO, > 99% purity) was obtained from sigma chemicals Co, USA. Ferulic acid ($C_{10}H_{10}O_4$, Molar mass: 194.18 g/mol) was brought from Acros Organics CO, USA. Selenium dioxide (SeO_2, Molecular weight: 110.96 was obtained from Sigma-Aldrich , part of Merck Company, USA. Ginger (*Zingiber officinale* rhizome dried roots was purchased from the local market, and grinded to a powder).

Ginger extraction preparation

The extract of *Zingiber officinale* rhizome dried roots extract was used to prepare selenium nanoparticles. About 20 gm of *Z. officinale* rhizome dried roots powder was immersed in a beaker containing 200 mL double distilled water and boiled for 30 min. The extract was cooled down and filtered with Whatman filter paper no.1 and the extract was stored at 4 °C [19].

Selenium nanoparticles preparation (SeNPs)

The cold water aqueous extract of ginger rhizomes obtained from the local market was used as a precursor for synthesis of SeNPs. Ginger extract (2 ml) was added drop wise into the 20-ml solution of SeO_3 (10 mM), with vigorous stirring. The mixture was incubated by placing the solution onto a rotatory orbital shaker operating at 200 rpm, 30 °C for 72 h in dark condition. The reduction of selenium ions was monitored by sampling an aliquot (3 ml) of the mixture at intervals of 24 h, followed by measurement of absorption maximum. Absorption maximum was determined by measuring optical density of the content from wavelength 350 to 700 nm using UV–Vis spectrophotometer [20].

Ferulic acid preparation

FA was dissolved in sterile water and administrated orally by gavage at a dose of 100 mg/kg body weight.

Experimental animals

Sixty Swiss Albino male rats (150-200 g) were drawn from the animal house of The Nile Company for Pharmaceuticals & Chemical Industries, Cairo, Egypt. They were maintained on a standard pellet diet and tap water. The animals were housed in suitable cages in conditioned atmosphere (20-22 °C) and they were allowed 7 days for adaptation.

Experimental design

Rats were divided into six groups:

Group 1 (control): Animals were administrated orally by gavage 1 ml of physiological saline.

Group 2 (SeNPs): Rats were administrated orally by gavage SeNPs (0.5 mg/kg body weight per day) for 21 days.

Group 3 (FA): Rats were administrated orally by gavage FA (100 mg/kg body weight per day) for 21 days.

Group 4 (AC): Rats were administrated orally by gavage with AC (50 mg/kg body weight per day) for 21 days.

Group 5 (AC+SeNPs): Rats were received AC as group 4 and then SeNPs as group 2.

Group 6 (AC+FA): Rats were received AC as group 4 and then FA as group 3.

Collection of samples

Brain tissue of experimental animals were dissected out after animal scarified and homogenized (10% w/v) in phosphate-buffered-saline (0.2 M sodium phosphate buffer with 0.15 M sodium chloride, pH 7.4) in a glass tissue homogenizer with Teflon pestle.

Determination of Monoamines content

The fluorometric assay for serotonin (SER), epinephrine (EPI) and norepinephrine (NE) levels determination was carried out according to Ciarolone [21]

Determination of Acetylcholinestrase (AChE)

Acetylcholinestrase (AChE) was determined in the brain tissue homogenate using the kite supplied by Abcam®, Acetylcholinesterase Assay Kit (Colorimetric).

Statistical analysis

SPSS version 20 was used for the statistical analysis. The data obtained were calculated by one- way analysis of variance (ANOVA). Difference was considered statistically significant at $P< 0.05$.

Results

Epinephrine (EPI) level in the brain tissue

EPI level (µg/gm) was determined in the brain tissue and results were represented in (table 1). Results showed that EPI level was significantly decreased ($P< 0.05$) in the AC group in compared to control group. On the other hand, EPI level was significantly increased ($P< 0.05$) in the AC group treated with SeNPs (AC + SeNPs) or FA (AC + FA) in compared to AC group.

Norepinephrine (NE) level in brain tissue

NE level µg/gm was determined in the brain tissue and results were represented in (table 2). Results showed that NE level was significantly decreased ($P< 0.05$) in AC group in compared to control group. On the other hand, NE level was significantly increased ($P< 0.05$) in AC groups treated with SeNPs (AC + SeNPs) or FA (AC + FA) in compared to AC group.

Table 1. Effect of SeNPs and FA on EPI level in the brain of AC treated rats.

	Control Mean±SE	SeNPs Mean±SE	FA Mean±SE	AC Mean±SE	AC+SeNPs Mean±SE	AC + FA Mean±SE
EPI (µg/gm)	48.67±2.9	50.40±4.1	50.72±2.7	31.38±2.5[a]	45.00±0.86[b]	43.22±1.07[b]

a: significantly ($P< 0.05$) different from the corresponding control group
b: significantly($P< 0.05$) different from the corresponding acrylamide group
Values are expressed as means ± SE. $p <0.05$.

Table 2. Effect of SeNPs and FA on NE level in the brain of AC treated rats.

	Control Mean±SE	SeNPs Mean±SE	FA Mean±SE	AC Mean±SE	AC+SeNPs Mean±SE	AC + FA Mean±SE
NE (µg/gm)	643.98±12.3	644.25±12.3	643.02±15.2	451.98±29.9[a]	634.58±13.6[b]	635.88±12.2[b]

a: significantly ($P< 0.05$) different from the corresponding control group
b: significantly($P< 0.05$) different from the corresponding acrylamide group
Values are expressed as means ± SE. $p <0.05$.

Serotonin (SER) in brain tissue

SER level (µg/gm) was determined in the brain tissue and results were represented in (table 3). Administration of AC significantly (P<0.05) decreased SER level in the tested brain tissue when compared to control group. On the other hand, SER level was significantly (P<0.05) increased in AC groups treated with SeNPs (AC+SeNPs) or FA (AC+FA) when compared to AC group.

Acetylcholine esterase (AChE) in brain tissue

AChE level (mU/ml) was determined in the brain tissue and results were represented in (table 4). Results showed that AChE level was significantly increased (P< 0.05) in AC group compared to control group. On the other hand, AChE level was significantly decreased (P< 0.05) in AC groups treated with SeNPs (AC + SeNPS) or FA (AC + FA)compared to AC group.

Table 3. Effect of SeNPs and FA on SER level in the brain of AC treated rats.

	Control Mean±SE	SeNPs Mean±SE	FA Mean±SE	AC Mean±SE	AC+SeNPs Mean±SE	AC + FA Mean±SE
SER (µg/gm)	3.94±0.22	3.68±0.32	4.19±0.26	2.28±0.20[a]	4.08±0.21[b]	3.47±0.15[b]

a: significantly (P< 0.05) different from the corresponding control group
b: significantly(P< 0.05) different from the corresponding acrylamide group
Values are expressed as means ± SE. p <0.05.

Table 4. Effect of SeNPs and FA on AChE level in the brain of AC treated rats.

	Control Mean±SE	SeNPs Mean±SE	FA Mean±SE	AC Mean±SE	AC+SeNPs Mean±SE	AC + FA Mean±SE
AChE (mU/ml)	13.90±0.4	13.73±0.7	14.43±0.3	54.05±5.2[a]	19.93±0.7[b]	31.62±3.2[b]

a: significantly (P< 0.05) different from the corresponding control group
b: significantly(P< 0.05) different from the corresponding acrylamide group
Values are expressed as means ± SE. p <0.05.

Discussion

AC is used in many fields, from industrial manufacturing to laboratory work. It is also formed during heating process through the interaction of amino acids. Therefore, AC poses significant risk for both human and animal health [22]. The present study indicated the neurotoxic effects of AC (50 mg / kg for 21 days) and its effects on the neurotransmitters (EPI, NE, SER) and also in the AChE activity. Efficient communication among large numbers of brain cells (i.e., neurons) is necessary for the normal functioning of the nervous system. A central mechanism of neuronal communication involves the release of neurotransmitters that bind to specialized receptors on the target cell, changing its activity [23]. Results from the present study showed that; intoxication of rats with AC produced significant decrease in the EPI, NE and SER contents in tested brain. Free radicals are generally produced *in vivo* and there are numbers of protective antioxidant enzymes (superoxide dismutase, catalase, glutathione *S* transferase, glutathione peroxidase and antioxidant glutathione) for scavenging it. Intoxication with AC induces oxidative stress and this is due to the ability of AC to produce ROS which leads to oxidative damage in the brain tissue. The over-production of ROS decreases the antioxidant system and increases the lipid peroxidation [24]. So, it affects some enzymes and signal pathways. AC is able to react with many intercellular molecules that contain –SH, -NH_2 or –OH. Many proteins involved in the release of neurotransmitters are thiol- rich and therefore, it is easy to form AC adduction. So, neurotransmitter release has been shown to be highly sensitive to inhibition by thiol alkylating chemicals such as AC [25]. Based on this, we can suggest that AC adducts the thiol groups on key regulatory proteins which control the production of neurotransmitters. Goldstein [26] suggested that defective neurotransmission in AC-intoxicated laboratory animals might be mediated by changes in the transmitter synthesis, strong uptake and release [27]. LoPachin et al [28] suggested that AC impaired neurotransmitter uptake into striatal synaptic vesicle. Waggas and Balawi [29] found that daily administration of AC for four weeks caused significant decrease in EPI and NE in the tested brain. Another study reported that AC interacts with tyrosine, which is the precursor amino acid for the synthesis of

NE [30]. Availability of monoamine neurotransmitters is regulated both by their synthesis and catabolism. EPI, NE and SER are the most important monoamine neurotransmitters in the brain. One of the major enzymes involved in the catabolism of monoamine neurotransmitters is monoamine oxidase (MAO) [31]. Brain MAO catalyses the oxidative deamination of a variety of amine neurotransmitters and then the byproduct H_2O_2 will be produced. H_2O_2 is considered to be one of the most important sources of oxidative stress and induced physiological peroxidation. Ghareeb et al [32] reported that AC increased the catalytic activity of MAO in the brain of rat. This might support that active degradation might cause the decreased brain monoamine level in AC-treated rat. From the present study and the previous studies it could be concluded that, AC caused a significant decrease in EPI, NE and SER in brain tissue; this may be due to axonal and nerve terminal degeneration which caused alternations in the synthesis of transmitter, storage uptake, release and reduction in synaptic vesicle and as a result, the content of EPI and NE decreased. AChE is a significant biological component of the membrane that contributes to its integrity and changes in permeability occurring during synaptic transmission and conduction [32]. Over-activation of AChE leads to faster acetylcholine degradation and consequently lowered stimulation of acetylcholine receptors, which cause a reduction of diverse cholinergic and non-cholinergic function [33, 34]. A decrease in acetylcholine levels in the synaptic cleft contributes to progressive cognitive impairment and possibly other neurological dysfunctions as seen in diabetic neuropathy or other neurodegenerative disorders [33, 35]. An increase in AChE activity has been found to inhibit cell proliferation and promote apoptosis [36]. AC induced significant elevation of neurotransmission markers such as AChE activity levels in the brain. The observed increase in AChE activity in AC-administrated rats are potential indicators of neuronal damage by oxidative stress induced by AC. Evidence suggested that the enhanced activation of AChE causes a reduction of cholinergic neurotransmission and affects other related functions such as cell proliferation and promote apoptosis. In accordance, several studies indicated that AC induced significant increase in the level of AChE in the brain of rats. [37, 38]

Selenium (Se) is a dietary essential trace element for humans. Selenium can be incorporated into selenoproteins in the form of selenocysteine and selenomethionine. It is also necessary for Se-containing enzymes, such as glutathione peroxidase. Therefore, Se plays a key role in antioxidation functioning [39]. Selenium is involved in conservation of functional brain activity and protects against the oxidative stress-related brain disorders [40]. The present study indicated the therapeutic effect of SeNPs in AC-induced neurotoxicity in rats. The present study showed that treatment with SeNPs significantly increased the level of neurotransmitters (EPI, NE, and SER) in the AC-intoxicated group. Selenium is a potent antioxidant acting on decreasing the brain oxidative stress and lipid peroxidation. By preventing the physiological peroxidation, the brain MAO activity decreased and this can lead to increasing the level of monoamine brain neurotransmitters (EPI, NE, SER). Many previous researchers indicated the relation between Se and MAO inhibition [41, 42]. In the present study, it was found that Se increased the level of monoamine neurotransmitters by minimizing the action of AC-oxidative stress, which is responsible for the increase of the MAO activity. On the other hand, a treatment of AC-intoxicated group with SeNPs significantly decreased the level of AChE in the brain tissue. AC intoxication is associated with defects on cholinergic neurotransmission [30, 31]. Acetylcholine is a neurotransmitter required for proper functioning of cholinergic transmission process [43]. According to the present study, Se could modulate the cholinergic system by inhibiting the activity of AChE in the brain of rat as it is shown in many previous studies [44-46]. Ferulic acid (FA) is an important compound of widely used medicinal herbs and belongs to the family of hydroxycinnamic acid. It has been credited with many pharmacological properties including neuronal progenitor cell proliferation, anti-inflammatory, antioxidant, and neuroprotective activities [47]. The present study showed the therapeutic effects of FA against AC-induced neurotoxicity in rats. The treatment with FA remarkably reversed the effects of AC-oxidative stress on the neurotransmitters. FA significantly increased the level of SER and NE and non- significantly increased EPI in the AC-intoxicated group. This finding indicates the fact that the FA is a potent antioxidant so it may reduce the monoamine decomposition by reducing the brain oxidative stress. The preservation of monoamine transmitters can be achieved either by inhibiting their reuptake or inhibiting their metabolism through MAO [48-51]. FA significantly decreased the level of AChE in the AC-intoxicated group. AChE is an enzyme relevant to cognitive function and

memory, and it has been shown to be therapeutic targets in the management of several neurodegenerative diseases, especially AD. Therefore AChE inhibitors may be a good approach toward the treatment and management of neurodegeneration [52]. AChE inhibitors decrease the hydrolysis of acetylcholine to elevate the endogenous level of acetylcholine in the brain and to boost cholinergic neurotransmission. The administration of FA led significantly to reversal in the AChE activity by decreasing the brain oxidative stress and could further corroborate the neuroprotective potential of FA as reported in many previous studies [53-56]. This study has been able to show the potential effect of FA at a dose of 50 mg/kg body weight and SeNPs synthesized by ginger extract at a dose of 0.5 mg/kg body weight showed a significant elevation in monoamine neurotransmitters and reduction in AChE activity. From a biochemical view, this study showed that SeNPs has potent therapeutic activity in comparison with FA.

Conflict of Interest

We declare that we have no conflict of interest.

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