·Basic Research·

Effectiveness of selenium on acrylamide toxicity to retina

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Abstract

• AIM: To investigate the hematological parameters, biochemical and electrophysiological role of acrylamide (ACR) in the retina and to assess whether selenium (Se) has protective potential in experimental oral intoxication with ACR.

• METHODS: Sixty Wistar age matched-albino rats (3mo) weighing 195-230 g comprised of both sex were divided into 4 groups. Group I served as the control one in which animals take saline; group II was animals administrated ACR in dose of 15 mg/kg body weight per day for 28d; group III was animals received ACR then additionally Se (0.1 mg/kg body weight) for 28d; and group IV was animals received Se only (0.1 mg/kg body weight) for 28d. Blood analysis and serum trace element levels (Fe, Cu, and Zn) were measured. The electroretinogram (ERG) was recorded, the levels of malondialdehyde (MDA) and glutathione peroxidase (GSH-Px) in the retinal tissues were determined. Moreover the regulation of ion channels such as calcium, sodium and potassium were studied. All measurements were done for all groups after 28d.

• RESULTS: Administration of ACR in group II caused a significant decrease (P<0.05) in hemoglobin (Hb), red blood cells (RBCs), hematocrit (HCT), white blood cells (WBCs) and lymphocyte of rats. A significant decrease (P<0.05) in Zn level, and alkaline phosphatase enzyme was observed compared to control. ERG which is a reflection of the electric activity in the retina; a- and -b wave amplitudes in ACR group had a reduction of 40% and 20% respectively. These changes accompanied by significant increases (P<0.05) in MDA level in the ACR group, in contrast with GSH-Px which is significant decreased (P<0.05). Moreover sodium and calcium were significant increased but potassium was significant decreased (P<0.05) compared to control group. There were no significant differences between group III (treated with Se) and control in all hematological parameter. Also serum trace elements levels (Cu, Fe and Zn), alkaline phosphatase enzyme and electric activity of the retina didn't change compared to control due to Se treatment.

• CONCLUSION: This study provides evidence for the protective effect of Se on acrylamide induced toxicity by reducing oxidative stress.

• KEYWORDS: selenium; acrylamide; retina; electroretinogram; hemoglobin

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INTRODUCTION

Acrylamide (ACR) (CH_2=$CHCONH_2$) is a widely used industrial chemical. It is a white, odorless, crystalline solid at room temperature. It has been reported to be present in plant material such as potatoes, carrots, radish, lettuce, Chinese cabbage, parsley, onions, spinach, and rice paddy in sugar and olives [1,2]. It is found in carbohydrate-rich food prepared at high temperatures such as French fries and potato chips that are consumed by humans. Consumption of these foods may result in significant human exposure to ACR. The biological consequences of ACR exposure have chiefly centered on neurotoxicity ever since this effect was observed in humans occupationally exposed to this compound [3]. ACR neurotoxicity may be attributed to its higher affinity to form adducts with glutathione, proteins, and DNA directly or after metabolized to its epoxide, glycidamide (2,3-epoxy1propanamide), glycidamide was found to produce severe lesions [4]. In addition, the ability of ACR to form haemoglobin adduct led to dysfunction of oxygen transport causing hypoxia led to vascular disturbance[5]. ACR exposure was found to be linked to nerve terminal damage, reduction of the antioxidative capacity and oxidative stress in rat cerebellum [6-8]. ACR has also been shown to affect the visual system, massive degeneration, vacuolization and cell loss in the ganglion cell layer, as well as general reduction in retinal size were detected [9]. Light and electronic microscopic examination revealed pathologic changes in the retina and optic nerves consistent with chronic stages of ACR-toxicity. Axonal swelling and later degeneration in the more central optic tract were described in ACR intoxicated animals and the visual evoked response abnormalities have been reported in rats [10,11]. The effect of ACR on the electrophysiological

properties of retina showed that it reduced amplitude of electroretinogram (ERG) 30 Hz flicker stimulation, and a prolonged VEP N75 component. ERG 30 Hz primarily reflects retinal cone and adjoining bipolar cell activity located centrally in the visual field. Furthermore a reduced visual light sensitivity centrally in the visual field, with a possible component of reduced colour vision after exposure to ACR was shown[12,13].

Selenium (Se) is a trace element that is essential to good health but required only in small amount. In the last 10y, there has been intense interest in Se supplementation and its role in health [14]. Major dietary sources of Se are plant foods (provided the soil is not deficient in Se), animal kidneys, seafood, egg yolk and Brazil nuts. Besides, the soil Se level is reflected in the concentrations seen in plants [15]. Se is incorporated into proteins to make selenoproteins, which are important antioxidant enzymes [especially, glutathione peroxidase (GSH-Px)]. The antioxidant properties of selenoproteins help prevent cellular damage from free radicals. Free radicals are natural by-products of oxygen metabolism that may contribute to the development of chronic diseases[16]. Other selenoproteins help regulate thyroid function and play a role in the immune system [17]. Some studies have addressed the roles of selenoproteins in the eyes, with evidence suggesting that Se supplementation may have a role in preventing cataract formation and age-related maculopathy, also a posse of beneficial effect on diabetic retinopathy [18,19]. Glutathione (GSH) could be one of the primary events in ACR-induced neurotoxicity. Se as a component of GSH-Px significantly increased GSH and GSH-Px levels and can partially prevent the biochemical changes of the rats which received ACR. So the present study was carried out to investigate the toxic effects of ACR in the retina and investigate the protective role of Se in experimental oral intoxication with ACR to determine the possible antioxidant mechanisms.

MATERIALS AND METHODS

Materials Rats were randomly selected from the animal house facility at the Research Institute of Ophthalmology, Giza, Egypt. The experimental protocol was approved by the local ethical committee that applies ARVO statements of using animals in ophthalmic and vision research. Rats were kept in rat cages in well ventilated house, temperature of 27℃-30℃, 12h natural light and 12h darkness, with free access to tap water and dry rat pellet. All chemicals were purchased from Sigma Chemical Co. (St.Louis, MO, USA). ACR of highest purity 99.9% and the applied dose of 15 mg/kg body weight were dissolved in 0.2 mL saline solution [20]. A pilot study was done to Wistar age matched-albino rats (3mo) weighing 195-230 g comprised of both sex by measuring all parameters before treatment and we found no significant difference. Hence we randomly selected 60 rats and divided them into 4 groups. Group I was the control one in which animals take saline; group II was animals administrated ACR (15 mg/kg body weight per day for 28d); group III was animals received ACR then directly additionally Se (0.1 mg/kg body weight) for 28d; and group IV was animals received Se only (0.1 mg/kg body weight per day) for 28d. Experiment was designed for the dose of administrated ACR to be 15 mg/kg body weight and the duration of 28d to study sub acute toxicity of ACR as per Organization for Economic Cooperation and Development (OECD) guidelines. Also the dose of Se (0.1 mg/kg) was sufficient to reach a plateau activity for cellular GSH-Px and non toxic dose. All medications were given orally by gastric intubation in the morning (between 09:00 a.m. and 10:00 a.m.).

Blood Analysis At the expiration of the experimental period (28d of continuous administration of ACR for group II, ACR+Se for group III and Se only for group IV), fasted control and other three groups were anesthetized under diethyl ether; the blood was collected from orbital venous sinus in ethylenediaminetetraacetate (EDTA) containing tubes for determination of the levels of blood parameters [hemoglobin (Hb), red blood cells (RBCs), white blood cells (WBCs), hematocrit (HCT) and differential leucocytes count] or kept without anticoagulant at room temperature for 1h, then centrifuged at 3000 rpm/30min. The non-hemolysed serum was obtained in clean sterilized rubber stoppered glass vials and stored at -20℃ until used for the serum trace element levels determination (Fe, Cu, and Zn).

Measurement of Serum Zn, Fe, Cu and Alkaline Phosphatase Microminerals or trace elements play a versatile function in human body ranging from developing immunity to provide antioxidant defence so, Serum Cu, Zn and Fe was measured as previously reported by Meret and Henkin [21]. Pure Cu, Zn and Fe solutions (Sigma Chemicals Co, St. Louis, MO, USA) were used as reference standard material. Five concentrations such as 20, 40, 60, 80 and 100 μg/dL for Cu and Zn, and 50, 100, 150, 200 and 250 μg/dL for Fe were prepared for standard calibration graphs for the respective minerals. For analysis of Cu and Fe, serum was diluted ×10 (9:1) with de-ionized water, and for Zn, serum was diluted 10× (9:1) with de-ionized saline water (0.82% NaCl). Hemorrhagic or cloudy serum was treated with 5% TCA and the clear supernatant was used. Absorbance for Cu, Zn and Fe were read at 324.8 nm, 213.9 nm and 248.3 nm respectively in the atomic absorption flame spectrometer (Perkin-Elmer, Wellesley, MA, USA). Alkaline phosphatase enzyme (ALP) was estimated by the method of Douglas *et al*[22].

Electroretinogram Photopic ERGs were recorded in the presence of white rod-suppressing back ground of 34 cd/m^2. All animals were light adapted for 10min before the

electrophysiological recording. They were anesthetized intraperitoneally ketamine and xylazine (100 and 5 mg/kg respectively), and after establishing the anesthesia, animals were placed on the pad of an operating table where their body temperature was maintained at 37℃. Each rat was positioned with its head resting to one side and local anesthetizing eye drops were also applied. The ERG was recorded by using three Ag-AgCl electrodes. The active electrode was a wick electrode placed at the corneal periphery. The other two electrodes were placed on the skin of the lower eyelid and on the ear, as reference and earthed electrodes, respectively. A white flash was used in this work with fixed intensity (4 lx) and duration (0.2s). The resulted electrophysiological signals were pre-amplified using CEPTU pream. (Bioscience, UK). The pre-amplified signals were delivered to a computer system attached to a digital oscilloscope (Velleman Co., Taiwan), to be recorded and analyzed by the provided software.

Measurement of Retinal Na^+, K^+, and Ca^{2+} Contents Our study emphasizes the importance of Na^+, K^+, and Ca^{2+} in the maintenance of ionic balance across cell membranes, because various clinical conditions are frequently complicated by vision loss through edema formation and apoptotic or necrotic cell death so, measurement of retinal Na^+, K^+, and Ca^{2+} were done. The eye was enucleated and rapidly opened; the retina was progressively detached from the retinal epithelium and finally dissociated by cutting from the optic nerve [23]. Retinal tissue was dried for 48h at 100℃ and then converted to ash at 550℃ for 24h. The ash was dissolved in 0.2 mL of 3 mol/L nitric acid and diluted 10-fold with deionized water. Tissue Na^+ was measured at a wavelength of 330.3 nm, K^+ at 404.4 nm, and Ca^{2+} at 422.7 nm in an air acetylene flame by an atomic absorption spectrophotometer (Perkin-Elmer, Wellesley, MA, USA) then determination of ion contents have been done as described previously in different tissue[24].

Malondialdehyde and GSH-Px Activity Measurements Oxidative stress has been demon strated to be key mechanisms in many ACR induced cell injuries and neurodegenerative diseases. Oxidative stress refers to enhanced generation of reactive species/reactive nitrogen species and/or depletion of antioxidant defence system causing an imbalance between pro-oxidants and antioxidants leading to apoptosis. Malondialdehyde (MDA) is a biomarker to measure the level of oxidative stress in an organism and GSH-Px is an enzyme whose main biological role is to protect the organism from oxidative damage. The eyes enucleated and retinas carefully isolated, the specimens were harvested and stored at 20℃ until biochemical assay. Frozen retinas were homogenized (Awel model MF 20/20-R-France) (1:10, w/v) in 50 mmol/L phosphate buffer (pH 7.4) and kept in an ice bath. The homogenate was then centrifuged at 5000 g for 30min to remove debris. The homogenate and supernatant were stored at 20ºC in aliquots until used for biochemical assays. The protein content of the tissue was determined by using the Lowry method[25].

MDA levels, an indicator of free radical generation that increases at the end of the lipid peroxidation, were estimated by the double heating method of Draper and Hadley [26]. The principle of the method is the spectrophotometric measurement of the color generated by the reaction of thiobarbituric acid (TBA) with MDA. The level of MDA is expressed as nanomoles per gram protein.

GSH-Px (E.C. 1.6.4.2) activity was measured by the method of Paglia and Valentine[27]. The enzymatic reaction in the tube that contained reduced nicotinamide adenine dinucleotide phosphate, reduced glutathione, sodium azide and glutathione reductase was initiated by the addition of hydrogen peroxide (H_2O_2), and the change in absorbance at 340 nm was monitored by a spectrophotometer. Activity was given in units per gram protein.

Statistical Analysis Data were represented as the mean±SD. For comparison between multiple groups the analysis of variance (ANOVA) procedure was used, where a commercially available software package (SPSS-11, for windows) was used and the significance level was set at $P<0.05$. For more statistical adjustment to avoid the possibility of type I error, Bonferroni correction was applied where the significant level of the ANOVA test ($P<0.05$) was lower to 0.00625.

RESULTS

As shown in Table 1, administration of ACR (15 mg/kg body weight); for 28d caused a significant ($P<0.05$) decrease in Hb, RBCs, HCT, WBCs, lymphocyte, monocytes and eosinophils of rats as compared to their corresponding controls. For group III that treated with Se after ACR administration, no significant differences between all hematological parameters measured values and control was observed. Also the same phenomena appeared to group IV which treated with Se only.

Table 2 showed serum trace elements levels (Cu, Fe and Zn) in microgram per liter and alkaline phosphatase enzyme in U/L. The ANOVA test showed there is no significant difference between control values neither with ACR group nor ACR+ Se group in Cu and Fe levels. On the other hand there was a significant decrease in Zn level, alkaline phosphatase enzyme after ACR administration and returned to their normal values after Se treatment.

Figure 1 (left panel) ERG records for all the studied groups where cursors 1 and 2 point to a and b wave respectively. Amplitudes were measured from baseline to the lowest point of the negative peak for the a wave and from the trough of

Table 1 Effect of oral administration (15 mg/kg b.wt.) for 28d on the level of blood parameters (Hb, RBCs, WBCs, HCT and differential leucocytes count) of Albino rats $\bar{x} \pm s$

Parameters	Control (group I)	Acrylamide (group II)	Acrylamide+Se (group III)	Se (group IV)
Hb (g/dL)	14.6±0.4	13.8±0.2[a]	14.3±0.4	14.8±0.5
RBCs (10^6/mm^3)	5.2±0.3	4.5±0.3[a]	4.9±0.5	4.8±0.6
HCT (%)	45.3±2.7	41.4±3.1[a]	43.8±2.4	44.4±2.5
WBCs (10^3/mm)	8.9±0.9	7.3±0.6[a]	7.9±1.5	8.5±1.2
Lymphocytes (%)	66±3.7	51±5.8[a]	62±6.4	63±4.3
Monocytes (%)	4±0.07	3±0.06[a]	3.9±0.1	4±0.05
Eosinophils (%)	2±0.02	1±0.03[a]	2±0.01	2±0.01
Basophils (%)	0	0	0	0

[a]Statistically significant.

Table 2 Serum copper, Zn, ferrous and alkaline phosphatase enzyme levels for all studied groups $\bar{x} \pm s$

Parameters	Control (group I)	Acrylamide (group II)	Acrylamide+Se (group III)	Se (group IV)
Cu (μg/L)	215±35	190±30	200±24	207±26
Fe (μg/L)	329±48	350±44	288±50	290±49
Zn (μg/L)	128±26	82±13[a]	125±17	123±25
ALP (U/L)	127±20	57±9[a]	117±10	125±5

[a]Statistically significant.

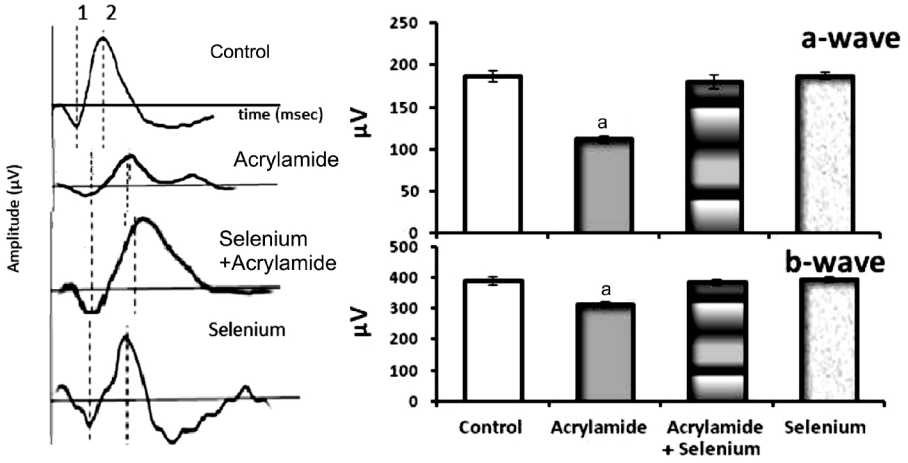

Figure 1 The left panel ERG records for all groups where the cursors 1 and 2 points to a and b wave respectively and the right panel histogram changes in amplitudes of a and b wave (μV) of ERG measurements for all studied groups Data expressed as mean±SD; [a]Statistically significant.

the a wave to the positive peak for the b wave. Figure 1 (right panel) illustrated the changes of a and b wave amplitude of ERG measurements for all the studied groups. The control amplitude of a wave which correspond to photoreceptor function was on the average 187±6.4 μV and the average b wave which reflect the electrical activity in the inner retina subsequent to photoreceptor stimulation was 392±12.7 μV. Statistically significant reduction in a and b waves was apparent in histogram for ACR group reflecting the effect of ACR administration where a and b wave found to be 112±4.7 μV and 313±10 μV respectively. After treatment with Se a and b wave amplitudes were 181±8 μV and 386±9 μV respectively and matches the control value. Also their values in group IV which treatment with Se only was not differ from the control values and found to be 188±4.4 μV and 395±7 μV respectively.

In the results obtained for biochemical assays of lipid peroxidation in the retinal tissue that given in histogram Figure 2, there were significant increases in MDA level in the ACR group compared with the control one (P<0.05), in contrast with GSH-Px which is significant decreased (P<0.05). There were no significant differences in MDA and GSH-Px level in rat's retina treatment with Se after ACR administration (group III) compared to control. Also the same phenomena appeared in group IV that treated with Se only.

The administration of ACR influence on the retinal cations content in the retina was illustrated in Figure 3. Sodium and calcium were significant increased in ACR group compared to control but potassium was significant decreased (P<0.05). After Se treatment (group III), all retinal cations concentration return to the control value range. There is no change in the concentration of potassium, sodium and

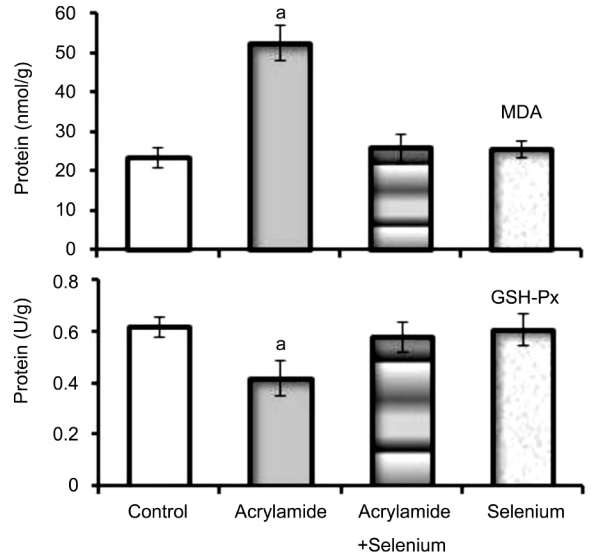

Figure 2 Changes of MDA and GSH−Px after application of acrylamide, Se and both compared to control in rat retinas Data expressed as mean±SD; [a]Statistically significant.

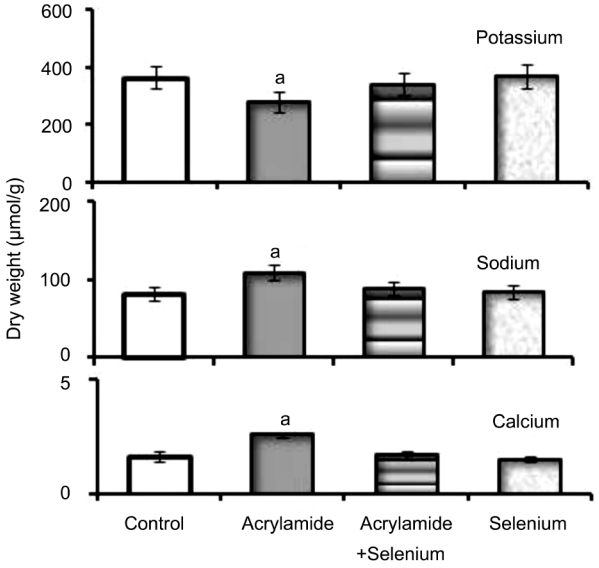

Figure 3 Effect of acrylamide, Se and both on cations (potassium, sodium and calcium) contents in rats retina Data expressed as mean±SD; [a]Statistically significant.

calcium in retina of rats treated with Se only (group IV) compared with control.

DISCUSSION

After consumption, ACR is absorbed in the circulation and distributed to various organs, where it can react with DNA, neurons, Hb, and essential enzymes [28]. In the present study, we provide experimental evidence in support of the role of oxidative stress in ACR toxicity and to assess whether ACR effects on hematological parameter and functional integrity of the retina can be ameliorated by co-treatment with Se.

In our study administration of ACR caused significant decrease in RBCs, Hb, HCT. These changes may be due to decrease in erythrocyte acid resistance, activation of lipid peroxidation and retarded synthesis or destruction Hb [29-32]. ACR is electrophilic and covalently binds to the cysteine residues and forms adducts with sulfhydryl groups on Hb resulting in the loss of heme part of Hb molecules thereby reducing the amount of Hb in the blood, which in turn may also be responsible for the anaemic conditions [33]. ACR and its epoxide glycidamide form adducts with (NH_2-terminal) NH_2 group of the valine residue of Hb, acrylamide form adduct with Hb so it leads to disturbance in serum iron concentrations[34]. The HCT depends on the erythrocyte mass, mean corpuscular volume and plasmatic volume. Generally, when the erythrocytes have a normal size, the modifications of HCT follow the red cell distribution width changes [35]. WBC count reduced following ACR treatment, this reduction could be due to their diminished production, redistribution from peripheral blood into the tissues or rapid destruction of WBC and ACR may be immunosuppressive[36].

Se induced changes in hematological parameters and effect on survivability of the RBCs [37]. Se deficiency is a factor in preventing the activity of GSH-Px and increasing the free oxygen radicals in the tissues such as RBCs, which leads to increase the oxidative damage of the tissues. Se changes the number of lymphocytes, and WBCs, that the type of body immunity system. Se leads to increase the number of the blood cells (WBCs and lymphocytes) and maintaining them in a high level for a longer period of time. Se deficiency is an effective factor on the reduction of the lymphocyte and weakness of immunity system[38,16].

The depletion of Zn in rats treated with ACR may be related to the retinal abnormalities. Zn is an integral part of SOD-containing enzyme, superoxide dismutase deficiency Possibly leading to decreased efficacy in glutathione reduction[39,40]. Experimental and clinical evidence indicate the importance of Zn in the maintenance of normal visual function [41]. Zn deficiency may complicate the clinical features, affect adversely immunological status, increase oxidative stress and the generation of inflammatory cytokines [42]. Moreover enhances lipid peroxidation and impairs the metabolism of amino acid, nucleic acids, and proteins [43,44]. Accordingly alkaline phosphatase activity, which is one of the best indicators of Zn content of the organism, decreased significantly after acrylamide administration.

ERG is a reflection of the electric activity in the retina; a and b wave amplitudes in ACR group had a reduction of 40% and 20% respectively with respect to the control value. This important reduction in wave's amplitude matches others studies which proved that a possible slight change in the ERG suggests subclinical effects on photoreceptors (cones) in the central part of the retina. The photoreceptors are particularly susceptible to oxidative damage because of their high polyunsaturated fatty acid content and high metabolic rate[12,13].

The biochemical characteristics of retinal tissue showed that

the GSH-Px activity level was significantly decreased due to ACR administration, but MDA was higher than those of control. ACR- induced neurotoxicity may be associated with the enhancement of lipid peroxidation and reduction of the antioxidative capacity[7]. MDA is a main degradative product of lipid peroxidation. It may indirectly represent the level of lipid peroxidation. GSH is a major intracellular antioxidant, as well as an important component in the metabolism of many xenobiotics, including ACR. Cellular oxidative stress can either lead to a depletion of GSH and apoptosis. Therefore glutathione-ACR adduct formation can favor cellular oxidative stress, which may be one possible mechanism governing ACR toxicity[45].

The basic levels of retinal Na^+, K^+, and Ca^{2+} contents in treated retinas with ACR were changed. There was a significant increase in retinal Na^+ and Ca^{2+} contents from control levels and tissue K^+ was reduced from the control value. Acrylamide exerted oxidant stress and deterioration effects on enzyme activities and lipid peroxidation, each can influence adversely the ability of membranes (sarcolemmal, sarcoplasmic reticular or mitochondrial) to control ion movements between cellular compartments. Most investigators attribute the injurious effects of oxidant stress to lipid peroxidation and consequent changes in membrane permeability and involve changes in the redox state of thiol groups which control the activity of enzymes and membrane protein[23].

The retina also has relatively low peroxide scavenging capabilities as shown by low glutathione concentration and GSH-Px activity. These properties in addition to its high Zn concentration suggest that the retina may be an ideal tissue in which to study the antioxidant effects [41]. Our data suggest that the retina is affected by the acrylamide administration and protective effect of Se on retina was determined by normalization of the level of Zn in serum and alkaline phosphatase activity in ACR+ Se group[39,46].

When Se was supplemented concomitantly with ACR, rats produced a recovery up to the control values in a and b waves amplitudes, MDA levels and GSH-Px thus suggesting a possible treatment to avoid damage caused by oxidative stress as a result of ACR administration. Our finding that lipid oxidative damage is increased in the GSH-Px deficient retina, most likely because of the dysfunctional enzymatic antioxidant pathway[47].

After treatment with Se there was a significant decrease in cellular Na^+ and Ca^{2+} contents and K^+ loss were significantly reduced to match the control leading to a significant reduction of edema formation and mitochondrial calcium-overload-induced cell death, which is a key mediator or signal of necrosis and/or apoptosis. Se treatment protects mitochondrial functional performance by preserving mitochondrial membrane potential and the activities of mitochondrial complexes [48,49]. In conclusion, acrylamide caused many adverse effects in the tissues reflected in significant increase in lipid peroxidation. The decrease in glutathione levels and GSH-Px activity might be one of the primary events in the ACR-induced hematological and retinal lesions. The administration of Se, as a component of GSH-Px in combination with ACR significantly lowered lipid peroxidation, and enhanced glutathione levels. To prove this hypothesis, intervention with glutathione and glutathione peroxidase should be further investigation.

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