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Toxicological Effects of Fried Potato Chips Supplementation on Young Rats

Abdullah S Alrethaia*

Department of Basic Health Sciences, College of Applied Health Sciences in Ar Rass, Qassim University, Al Qassim, Al Rass, Saudi Arabia

***Corresponding Author**: Abdullah S Alrethaia, Department of Basic Health Sciences, College of Applied Health Sciences in Ar Rass, Qassim University, Al Qassim, Al Rass, Saudi Arabia.

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Abstract

The present study was carried out to explore the effects of fried potato chips supplemented diet on growth parameters and histopathology of gastrocnemius muscle in juvenile rats. Acrylamide was quantified in fried potato chips by HPLC method. To study the effect of fried potato chip (FPC) supplementation rats were randomly divided in to three groups and served as Control, ACR-treated (30 mg kg-1 BW) and fried potato chips (FPC) - fed (30% of diet). After 35 days of treatment, growth parameters like body weight and head rump length were measured. The animals were then sacrificed, length of the femora was measured; gastrocnemius muscle was excised and fixed paraffin for histopathology. Fried potato chips were found to contain significant amounts of acrylamide (4000 - 12500 ug kg-1). Administration of fried potato chips and acrylamide to young rats resulted in severe neurological disturbances including ataxia. The severities of the clinical signs increased with time and were more pronounced in ACR-treated group compared to the FPC-fed group. The results indicated a significant decrease in body weight gain, head-rump and femur growth of ACR-treated and FPC-fed rats compared to their control rats. Microscopic examination of Haemotoxylin and Eosin stained sections of the gastrocnemius muscle of the control rats revealed normal skeletal muscle architecture with normal endomysium, perimysium and muscle fibers. However, in the experimental groups several myopathic changes were observed wherein, the gastrocnemius muscle of both FPC-fed and ACR-treated rats exhibited thickening of the endomysial and perimysial connective tissue sheaths. The findings warrant further epidemiologic investigations to assess the health effects of consumption of fried potato chips in the world population.

Keywords: *Acrylamide; Toxicity; Young Rats; Fried Chips; Safety*

Abbreviations

ACR: Acrylamide; FPC: Fried Potato Chips; H & E: Haemotoxylin and Eosin

Introduction

Acrylamide (ACR) is an alpha, beta-unsaturated vinyl monomer of poly-acrylamide with very high water solubility. The harmful effects of ACR were generally discussed and researched with respect to its occupational exposure as an industrial material until Swedish National Food Administration and Stockholm University reported the unexpected presence of alarming amounts of acrylamide in a range of heat-processed foods in 2002 [1-5].

Exposure to ACR can occur as a consequence of environmental contamination with its use in soil sewer grouting or in waste paper recycling [6] and active or passive exposure to cigarette smoke [7]. These exposures can easily be identified and corrected. However, the finding that ACR is formed by itself in fried and oven-dried foods makes it a timely topic for researchers and clinicians involved in studying the impact of food contaminants on human health [8]. For the general population acrylamide-induced health hazards are based on

an average exposure to 4 ug/kg bw/day. Exposures to 200 - 300 ug ACR/kg bw/day are reported to put humans at risks for neurotoxicity and carcinogenicity [9,10].

Dietary ACR is largely derived from heat-induced reactions (Millard reaction) between the predominant amino group of the free amino acid precursor, asparagine, and the carbonyl groups of glucose and fructose. This occurs during heat processing (baking and frying) of plant-derived foods; especially starchy ones. Experimental studies revealed that temperatures higher than 100°C are enough for acrylamide formation in starchy foods [11-14]. Researchers also pointed out that extending the cooking period and/or increasing the cooking temperature results in more acrylamide formation in cooked foods [15].

Humans are continuously exposed to ACR through the diet, beginning with in utero exposures. In pregnant women, 10 - 50% of dietary ACR has been found to cross through the placental blood to the fetus [16,17]. ACR exposure continues through breast-feeding [18,19] and the consumption of baby food [20]. Breast milk has been found to contain ACR up to 18.8 mg/L [18]. Dietary exposure to acrylamide continues during childhood, adolescence and adulthood through the consumption of fried starchy foods. WHO estimated the human daily intake of ACR to be 1 - 4 mg/kg of body weight [21]. It is expected that infants and children have intakes that are two to three times those of adults when expressed on a body weight basis [22].

According to the Swedish Food Administration, fried potato products and bread contributed most to the dietary exposure of acrylamide [24]. A number of researcher have reported the presence of very high levels of ACR in fried potato products [24-26] due to the presence of high levels of acrylamide precursors, namely, reducing sugars and asparagine. It is estimated that asparagine constitutes 40% of total amino acid content of potatoes [27]. Recent studies revealed that the amount of ACR in fried potato chips, which rank number one snack both in industrialized nations and developing countries [28], was more dramatic in comparison to all other forms of heat processed potatoes, including French fries. This was attributed to the greater surface area-to-volume ratio of thin potato slices used for potato chip production which promotes rapid formation of acrylamide during frying [29,30].

The growth and development of rodents. El- Sayyad., *et al.* showed that regular consumption of fried potato chips during pregnancy and lactation in rodents caused delayed growth, reduced body and brain weight, decreased crown-rump length, myodegeneration, neurotoxicity and skeletal growth retardation of their offspring [31].

Nowadays it is possible that acrylamide toxicity is higher in children than any other age group, as chronic daily contact with acrylamide through consumption of fried starchy foods mainly potato chips is virtually inevitable [28]. To our knowledge, data concerning the effects of chronic exposure to dietary acrylamide in fried potato chips on children are scare. In view of this, the present study was planned to investigate the effects of fried potato chips supplemented diet in juvenile rats on their growth parameters, skeletal muscles' architecture and neuro-behavior; to address the potential health hazards associated with the possible ACR toxicity caused by the consumption of fried potato chips in children.

Materials and Methods

Chemicals and reagents

Acrylamide and methanol were purchased from Sigma Aldrich (ST. Louis, MO, USA). Standard pellet diet consisting of 50% ground barley, 10% ground yellow maize, 20% milk powder, and 10% vegetable oil were purchased from the Animal House of the Faculty of Pharmacy, King Saud University, Saudi Arabia. All the other chemicals and reagents used in the study were of extra pure analytical grade.

Estimation of acrylamide in potato chips

Stock solution of 1000 µg mL^{-1} acrylamide was prepared in double distilled water and diluted to prepare100 µg mL^{-1} working standard solution. The solution was stored at 4°C and used within 1 month.

The fried potato chips purchased from a local market were finely ground using warring blender. The chips samples (4g) were then defatted by adding hexane (10 mL) and shaking for 10 minutes. The mixture was then filtered and residue was dried under vacuum. Acrylamide was extracted by adding 20 mL of acetone and 100 µl of water to the defatted residue in a conical flask and placed in an ultrasonic bath at 40°C. After 20 min the extract was filtered through Whattman No 1 filter paper. 10 mL of the filtrate was evaporated under

vacuum and re-dissolved in 2 mL of double distilled water. The aqueous was filtered through membrane filter and 20 µL of this solution was injected into the HPLC system equipped with reverse phase C18 column (2 × 250 mm), UV detector and an isocratic pump. Water was used as mobile phase; column oven was set at 40 °C and analysis was performed at 202 nm [32].

Animals

One hundred and fifty young Albino rats (30 days old) of both sexes were obtained from the Animal House of the Faculty of Pharmacy, King Saud University, Saudi Arabia. The animals were allowed to acclimatize for 5 days to the laboratory conditions of the Animal House of Arras College of Applied Health Sciences, Qassim University prior to the commencement of the experiment on day 35. All of the experimental protocols were approved by the Animal Ethics Committee of Arras College of Applied Health Sciences and were according to the recommendations of Qassim University guidelines for the care and use of laboratory animals.

The rats were housed in plastic cages (40 × 25 × 16 cm) with 5 animals per cage. They were maintained under standard experimental conditions of 24 ± 2°C, 45 ± 5 RH, 12 hour photo period and provided with food and water *ad libitum*.

Experimental design

The rats were randomly divided into the following 3 groups each consisting of 50 animals:

1. **Group I (control group):** rats were fed with standard pellet diet.
2. **Group II (ACR-treated group):** rats were fed with standard pellet diet and received acrylamide (30 mg kg^{-1} bodyweight) dissolved in normal saline by gavage.
3. **Group III (FPC-fed group):** rats were fed with modified pellet diet containing 70% of standard diet and 30% fried potato chips.

The animals were maintained on the above treatments for 35 days. Throughout the study period the rats were observed once daily for one complete hour for nervous manifestations. Mortality was observed in terms of the day of the death and overall mortality rate.

Growth parameters

The body weight and head rump length (HRL) of the control and experimental were taken at the beginning [Day 35] and end [Day 70] of the experiment under anesthesia induced by (sodium pentobarbital; 40 mg/kg body weight i.p.) [33]. A single-pan electronic balance (Gebr. Bosch, Germany) having 0.1 mg accuracy was used to record the body weight. While, head rump length was measured from the dorsal surface of the animal's body using a vernier caliper. The distance noted was between the external occipital crest and the center of the anal opening of the rat [34].

At the end of the study period, surviving rats were sacrificed by cervical dislocation; their femora were dissected out and immersed in water overnight for easy separation of soft tissue. The length of the femora from the articular head to the medial condyles [35] was then measured by a vernier caliber to assess the effect of ACR and FPC supplementation on the linear growth of bones. The lengths of left and right limb femora were determined and their mean was taken as the bone length.

Histopathology

At the end of the study period, rats were sacrificed, and small portions of the gastrocnemius muscle were excised, washed with normal saline, fixed in 4% paraformaldehyde and embedded in paraffin. Longitudinal and transverse histological sections of 5 µm thickness was cut, stained with hematoxylin and eosin (H&E), and observed under a photomicroscope (Carl Zeiss, Germany).

Statistical analysis

All values are expressed as mean ± SD (Standard Deviation). The data were analyzed by ANOVA followed by Tukey's multiple comparisons test for significant differences using SPSS 20.0 computer software. Values were considered significant at $p \leq 0.05$.

Results and Discussion

Acrylamide concentration in fried potato chips

The fried potato chips were found to contain significant amounts of acrylamide. Potato chips were purchased from 10 different shops and 3 different time intervals. The results revealed that the chips contained 4000 - 12500 ug kg^{-1} of acrylamide. The average value was found to be 5500 ug kg^{-1}.

Clinical signs and mortality

In the present study, it was observed that administration of fried potato chips and acrylamide to young rats resulted in severe neurological disturbances including ataxia. The severities of the clinical signs increased with time and were more pronounced in ACR-treated group compared to the FPC-fed group. Increased landing of limbs was noticed from the 12th day in ACR-treated group and from 21st day in FPC-fed group. Additionally, splaying of the hind limbs (Figure 1) was seen in ACR-treated group on the 19th day of the treatment and the severity increased with time.

Figure 1: *A photograph of an ACR-treated rat showing splaying of both hind-limbs.*

In case of ACR-treated group, mortality commenced from the 10th day of ACR administration, while in FPC-fed rats, mortality commenced from the 25th day of administration. By the end of the experiment, mortality rate was found to be 24% and 4% in the ACR and FPC-fed groups, respectively. Post-mortem dissection of the rats revealed emaciation and generalized enlargement of body organs with hemorrhagic spots in the liver, spleen and kidneys. These changes were more obvious in the ACR-treated rats compared to FPC-fed rats.

Effect of fried potato chips and acrylamide on body weight, head-rump (HRL) and femur length of rats

In the present study, the effects of oral administration of ACR and FPC on the growth parameters of young rats were studied. The results indicated a significant decrease in body weight gain, head-rump and femur growth of ACR-treated and FPC-fed rats compared to their control rats (Table 1). The results also indicated that the growth suppression was more severe in ACR-treated rats than those of FPC-fed rats. At P_{35} (1st experimental day) the mean values of body weight, HRL and femur length of the rats were44.8g, 85.0 mm and 1.65 mm, respectively. By the end of the experiment (P_{70}) the body weight gain of the ACR-treated and FPC-fed rats was less than the control value by 51.5% and 35%, respectively. The rate of growth in the HRL of ACR-treated and FPC-fed rats was less than the control value by 53.2% and 23.5%, respectively, while the linear growth of their femur was less than the control value by 84.0%and 57.9%, respectively.

Treatment	Body Weight mean ± SD	Head-rump Length mean ± SD	Femur Length mean ± SD
Control	90.9^{c} ± 13.0	108.5^{c} ± 9.3	2.34^{c} ± 0.09
Fried Potato Chips	74.8^{b} ± 6.3	98.4^{b} ± 8.3	1.94^{b} ± 0.09
Acrylamide	67.2^{a} ± 10.6	93.2^{a} ± 10.4	1.78^{a} ± 0.1

Table 1: *Effect of fried potato chips and acrylamide on body weight, head-rump length and femur length of P_{70} rats after 5 weeks of treatment*

**Values carrying different superscript letters a,b,c in columns differ significantly at $p \leq 0.05$.*

Effect of fried potato chips and acrylamide on the histology of the gastrocnemius muscle of juvenile rats

Microscopic examination of H&E stained sections of the gastrocnemius muscle of the control rats revealed normal skeletal muscle architecture with normal endomysium, perimysium and muscle fibers (Figure 2A, 3A and 4A). However, in the experimental groups several myopathic changes were observed wherein, the gastrocnemius muscle of both FPC-fed and ACR-treated rats exhibited thickening of the endomysial and perimysial connective tissue sheaths (Figure 2B and 2C). In FPC-fed rats, extra-vasated RBCs were seen scattered between the individual muscle fibers (Figure 3B) but the occurrence of extra-vasated RBCs was even more severe in ACR-treated rats along with myodegeneration (Figure 3C). Furthermore, the sections of both FPC-fed and ACR-treated rats showed muscle fiber splitting, nuclear disintegration and increased incidence of centrally located nuclei. However, the effects were more severe in ACR-treated rats compared to FPC-fed rats (Figure $4C_1$ and C_2). Eosinophil, mast cells and plasma cells were seen in the sections of FPC-fed rats (Figure $4B_3$), while severe neutrophil infiltration was also seen in ACR-treated rats along with occurrence of eosinophil, mast cells and plasma cells (Figure $4C_3$). Furthermore, the striations of the muscle fibers in control rats were dark and uniform, while they were pale and not uniform in ACR and FPC-fed rats. Pronounced separation of muscle fibers was seen in ACR-treated rats compared to FPC-fed rats.

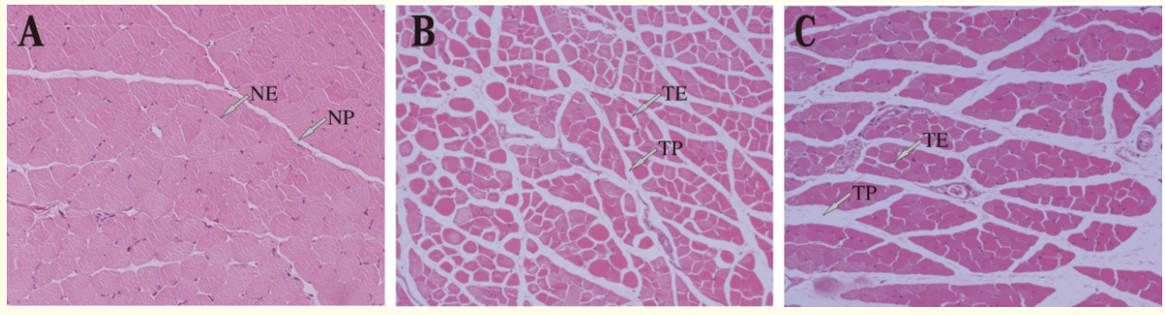

Figure 2: *Effect of fried potato chips and acrylamide on the histology of the gastrocnemius muscle of juvenile rats (T.S. at 200 x). A: Control, B: Fried Potato Chips, C: Acrylamide. NE: Normal Endomysium, NP: Normal Perimysium, TE: Thick Endomysium, TP: Thick Perimysium.*

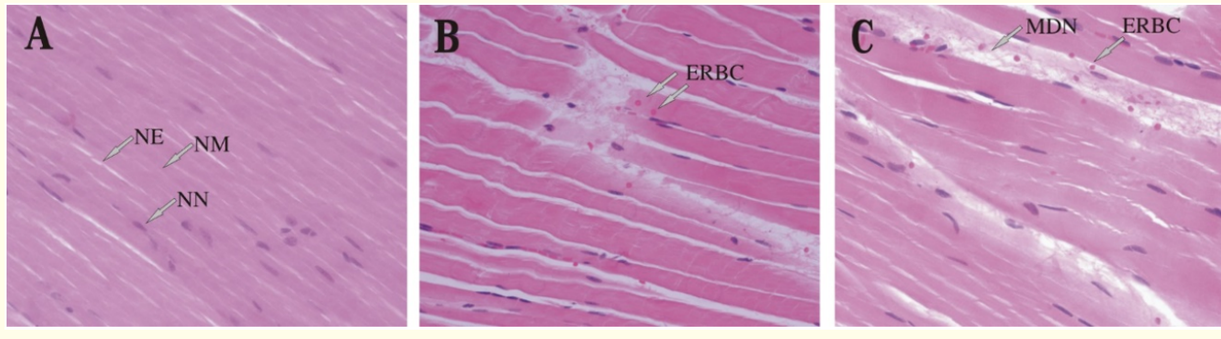

Figure 3: *Effect of fried potato chips and acrylamide on the histology of the gastrocnemius muscle of juvenile rats (L.S. at 400 x). A: Control, B: Fried Potato Chips, C: Acrylamide. NE: Normal Endomysium, NN: Normal Nucleus, NM: Normal Myofiber, ERBC: Extra-Vasated RBC, MDN: Myodegeneration.*

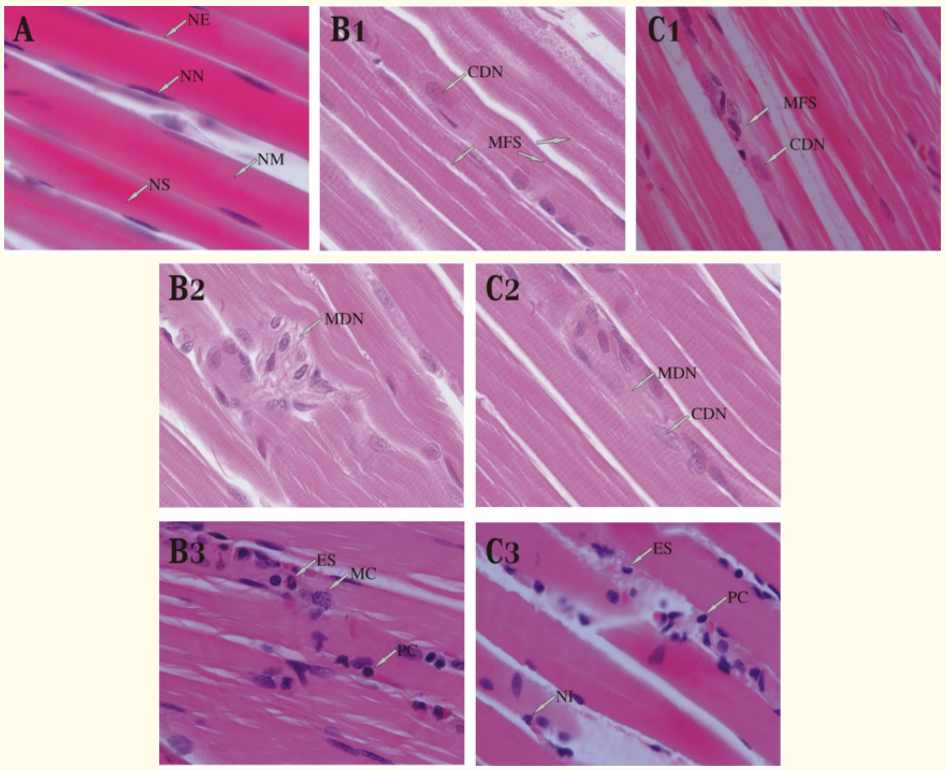

Figure 4: *Effect of fried potato chips and acrylamide on the histology of the gastrocnemius muscle of juvenile rats (L.S. at 1000 x). A: Control, B1-B3: Fried Potato Chips, C1-C3: Acrylamide. NP: Normal Perimysium, NS: Normal Striations, NN: Normal Nucleus, NM: Normal Myofiber, CDN: Centrally Located Degenerated Nucleus, MFS: Muscle Fiber Splitting, MDN: Myodegeneration, ES: Eosinophil, NI: Neutrophil Infiltration, MC: Mast Cell, PC: Plasma Cell.*

Discussion

Acrylamide (ACR) is a small organic neurotoxic molecule with high water solubility [31]. Although the toxicity and carcinogenicity of ACR is well established in both experimental animals and humans, ACR generated more scrutiny by the food scientists only after the Swedish National Food Administration and Stockholm University reported the presence of significant amounts of acrylamide in a range of heat-processed foods in 2002 [36]. ACR is formed primarily through Millard reaction occurring between amino acid (asparagine) and short chain sugars found in plant foods specially potatoes. With the current fast paced and convenience oriented lifestyle, the consumption of fast and fried foods including potato chips has increased tremendously. Although quantity of acrylamide ingested at a given point of time is insignificant, repeated exposure especially among young children can pose serious health risks.

It was found in the present study that ACR concentration ranged widely form 4000 - 12500 ug kg^{-1} among different fried potato chips samples acquired from different stores and at different times. Although these values are in good agreement with the earlier reports, the variation can be attributed to the frying temperatures as the rate ACR formation is directly proportional to the frying temperature [31].

The results of the present study revealed marked abnormal neuro-behavior, in both ACR-treated and FPC-fed rats, which was time dependent. These findings were parallel with LoPachin who determined a time of onset and a time-dependent progression of neurotoxicity in rats after ACR treatment [37]. Hind limb dysfunction and abnormal gait recorded in the present work coincide with Shukla., *et al.* who found that exposure of rats to ACR caused hind limb paralysis in 58% of the examined animals; they attributed these findings to ACR neurotoxicity [38].

The mortalities observed in the current study may be attributed to ACR neurotoxicity causing hind limb dysfunction which led to inability to get food as suggested by El-Bohi., *et al* [39]. In addition, ACR may cause alteration in the thirst and hunger regulation centers in hypothalamus [40].

This study also clearly demonstrated that both ACR and fried potato chips containing ACR caused significant growth suppression as evidenced by the reduction in body weight gain, HRL. In addition, there was a deleterious alteration of the architecture of the gastrocnemius muscle, which is involved in standing walking, running and jumping. These toxic effects of ACR might be attributed to its higher water solubility resulting in ready absorption from the oral cavity and also easy penetration into the cells [41]. Generally, two-third of the absorbed ACR is excreted in the urine and bile as metabolites with half-life period of few hours. However, the ACR or ACR metabolites bound to the proteins in the blood stream or within the cells may have a half-life of 10 days. Hence the regular exposure to ACR through food results inhibition of glutathione-S-transferase to produce glycinamide. Thus, binding of ACR with glutathione-S-transferase or glycinamide may interfere with cell function and induce cell death [42]. The ACR metabolite glycinamide has been shown to produce severe lesions in cerebral and peripheral nervous tissues [42].

Conclusion

Since, fried potato chips are the most popular and widely consumed snack products worldwide especially by young children and adolescents; there lurks great danger for the health of these individuals. Our study clearly demonstrated that ACR content in fried potato chips is unregulated due to the fact that the formation of ACR depends on a number of factors including concentration of asparagine, reducing sugars, frying temperature and duration of frying. Therefore, reducing the sugar content of potatoes by blanching, using low asparagine containing potato varieties and regulating the frying temperatures and duration could potentially reduce the ACR concentration in fried potato products. However, more efforts are required by the nutrition scientists to create awareness about the ill health effects of ACR among general public and food industries. In addition, strict law enforcement should be undertaken regarding food manufacturing practices and serious governmental measures should be taken to monitor food contaminants, particularly acrylamide. The findings warrant epidemiologic investigations to assess the health effects of consumption of fried potato chips in the world population.

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Conflict of Interest

The author declares that there are no conflicts of interest.

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