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Original Article

The impact of vitamin E against acrylamide induced toxicity on skeletal muscles of adult male albino rat tongue: Light and electron microscopic study

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ABSTRACT

Acrylamide, one of the major environmental public health problems, results from its increased accumulation in the process of cooking food materials. This study aimed to demonstrate the light and electron microscopic structural effects of acrylamide on the skeletal muscle fibers of adult male albino rat tongue and to investigate the possible protective effect of vitamin E co-administration. Thirty adult male albino Sprague–Dawley rats were divided into 3 groups, each group included 10 rats. Group I (control), group II which was subdivided into two equal subgroups: subgroup IIa: included 5 rats that received acrylamide orally once daily for 20 days. Subgroup IIb: included 5 rats that received acrylamide orally once daily for 40 days. Group III was also subdivided into two equal subgroups: subgroup IIIa: included 5 rats that received acrylamide and vitamin E orally once daily for 20 days. Subgroup IIIb: included 5 rats that received acrylamide and vitamin E orally once daily for 40 days. At the end of the experiment the tongue was dissected out for histological and electron microscopic studies, another muscle sample was homogenized and processed for biochemical estimation of malondialdehyde (MDA) and total antioxidant capacity (TAC). Light microscopic study of tongue skeletal muscles in acrylamide exposed animals revealed abnormal wavy course and splitting of the muscle fibers with fatty infiltration in between. Moreover, pyknosis and remnants of nuclei were detected. EM revealed marked aggregation of mitochondria of different size and shape with giant cells formation, and partial loss of myofilaments. There were statistically significant increase in MDA and decrease in TAC indicating oxidative stress in acrylamide administrated groups (group II) than the control group which increased by prolonged duration (subgroup IIb versus subgroup IIa, $p < 0.0001$). This oxidative stress could explain the histological changes in tongue muscles of acrylamide exposed rats. Co-administration of vitamin E with acrylamide ameliorated most of the above mentioned histological changes in the animals used and signs of improvement that became better with prolonged administration of it (subgroup IIIb versus subgroup IIIa, $p < 0.0001$) were detected. It could be concluded that, chronic exposure to acrylamide might lead to skeletal muscle damage in rat tongue which becomes worth with prolonged duration of exposure. Acrylamide induced oxidative stress is the implicated mechanism of such histological changes. This toxic effect of acrylamide could be minimized when vitamin E is given concomitantly with it by its antioxidant effect.

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1. Introduction

Acrylamide (ACR) is a low molecular weight, water soluble vinyl monomer from which polyacrylamides are synthesized to be used in the personal care and grooming products, such as lotions, cosmetics and deodorants [1]. It is commonly used in industries and laboratories. Its industrial application is associated with pollution and health risks. In addition, ACR is also, extensively used in molecular laboratories for gel chromatography [2]. Acrylamide has been reported to be present in plant material like potatoes, carrots, radish, lettuce, Chinese cabbage, parsley, onions, spinach and rice paddy [3] in sugar [4] and olives [5]. Major health concerns are associated with ACR due to its various sources and methods of exposure as in drinking water, inhalation, skin absorption and occupational exposure [6]. Direct exposure to acrylamide may result from ingestion of high-carbohydrate foods prepared at high temperatures such as potato crisps, crackers and French fries [7]. Indirect exposure may result from residual traces of the monomer in food packaging where polyacrylamide is used as a binding agent [8]. Acrylamide is formed in foods, if the heating frying is done in an oven, on a frying pan or by microwave heating, while no acrylamide has been detected in boiled food products [9,10]. It is generated from food components during heat treatment resulting from the reaction between the amino acid asparagines and reducing sugars (Maillard reaction) [11–13]. ACR has been reported to be neurotoxic [14,15], toxic to the reproductive system [16,17] and carcinogenic in experimental animals [18]. Although the polymer is nontoxic, occupational exposure of humans and experimental intoxication of laboratory animals with the monomeric form produces a neurotoxic syndrome characterized by ataxia, skeletal muscle weakness and weight loss [19,20].

Some studies demonstrated that acrylamide-induced cytotoxicity was relevant to oxidative stress [21]. The cytotoxic properties of acrylamide by affecting the cellular redox status may lead to generation of reactive oxygen species (ROS) causing cytotoxic and genotoxic effects [22]. Some strategies have been postulated to reduce acrylamide-mediated cytotoxicity by using natural antioxidant like vitamin E. Vitamin E can protect cellular structures against damage from free radicals such as peroxy radical, hydroxyl radical, as well as super oxide. Also, it has protective effect from oxidation products such as malondialdehyde (MDA) and hydroxynonenal. Vitamin E, as an important antioxidant, plays a role in inhibition of mutagen formation as well as repair of membranes and DNA [23].

So, the aim of the current study is to demonstrate the light and electron microscopic structural effects of acrylamide on the skeletal muscle fibers of adult male albino rat tongue and to investigate the possible protective effect of vitamin E co-administration.

2. Materials and methods

2.1. Animals

A total of 30 adult male albino Sprague–Dawley rats (200–250 g) were used in this study.

2.2. Used drugs

- Acrylamide (99.9 purity) was obtained from sigma chemical Co. Its molecular formula is C_3H_5NO. The applied dose of 15 mg/kg body weight [24] was dissolved in 0.2 ml saline solution and given orally by gastric tube.
- Vitamin E was obtained from sigma chemical Co. The applied dose of 100 mg/kg body weight [25] was dissolved in 1 ml of corn oil and given orally by gastric tube.

2.3. Experimental protocol

The experimental protocol of the study was approved by the ethical committee of Medical Faculty of Mansoura University. Animals were used in accordance with the Animal Welfare Act and Guide for Care Use of MERC (Mansoura Experimental Research Center) prepared by Mansoura University. The rats were housed in metal cages with meshes. They were maintained on commercial food and libitum consisting of standard laboratory rat chow and had free access to drinking water. All cages were kept at room temperature. Rats were divided into 3 groups, each group included 10 rats:

- *Group I*: Animals received 1 ml normal saline 0.9% orally by gavage and served as control.
- *Group II*: Animals were further subdivided into two equal subgroups:
  Subgroup IIa: included 5 rats that received acrylamide orally once daily for 20 days.
  Subgroup IIb: included 5 rats that received acrylamide orally once daily for 40 days.
- *Group III*: Animals were further subdivided into two equal subgroups:
  Subgroup IIIa: included 5 rats that received acrylamide and vitamin E orally once daily for 20 days.
  Subgroup IIIb: included 5 rats that received acrylamide and vitamin E orally once daily for 40 days.

At the end of the experiment the tongue was dissected out for histological and electron microscopic studies.

2.4. Histological study

Specimens from the tongue were fixed in Bouin's solution. After fixation, specimens were dehydrated in an ascending series of alcohol, cleared in two changes of xylene and embedded in molten paraffin. Sections of 5 microns thickness were cut using rotary microtome and mounted on clean slides. For histological examination, sections were stained with hematoxylin and eosin (H&E) [26].

2.5. Electron microscopic study

For transmission electron microscopy, small pieces of the tongue were fixed in 2.5% glutaraldehyde buffered with 0.1 M cacodylate (pH 7.2) for 2 h. The specimens were thereafter washed three times with the same buffer and post fixed in 1% buffered osmium tetroxide for 2 h then dehydrated in graded series of ethanol, infiltrated with epoxy resin mixture and embedded in epon–araldide

mixture. Ultrathin sections were cut by LKB ultratome and stained with uranyl acetate and lead citrate [27]. The ultrastructural analysis was carried out with transmission electron microscope in the Electron Microscopic Unit, in Faculty of Medicine, Tanta University.

2.6. Morphometric study

The degenerated fibers and pyknotic (shrunken deeply stained) nuclei were counted in 10 (non-overlapping) high power fields (HPF) at a magnification (×400) in H&E stained sections of T.S. of skeletal muscle of the tongue for each animal in all groups.

2.7. Determination of tissue malondialdehyde (MDA) and total antioxidant capacity (TAC)

To carry out the assays, the skeletal muscle of the tongue was weighed and homogenized by adding a 9 times of the volume of 0.9% saline. The 10% homogenate was centrifuged for 10 min (1800 g/min) and the supernatant was diluted with 10 times of the volume of 0.9% saline to 1% concentration. Malondialdehyde (MDA) as a marker of lipid peroxidation, was determined according to the thiobarbituric acid reaction described by Draper et al. [28]. One volume of sample was combined with two volumes of the stock reagent containing 15% trichloroacetic acid, 0.375% thiobarbituric acid and 0.25 M HCl. The mixture was kept in a boiling water bath for 15 min and after cooling, centrifuged at 1000 × *g* for 10 min. The absorbencies were measured at 535 nm against a reagent blank (Jenway, Genova Model, UK). Quantitations were obtained by using molar extinction coefficient of $1.56 \times 105\ M^{-1}\ cm^{-1}$. The results were expressed as nmoleMDA/g tissue.

Total antioxidants were colorimetrically determined according to the method described by Koracevic et al. [29] using the kit (Cat. No. # TA 2512) supplied by Bio-Diagnostics, Dokki, Giza, Egypt.

2.8. Statistical analysis

Data were expressed as mean value ± SD. Comparisons between two related groups were carried out by paired *t*-test while comparisons between more than two groups were carried out by analysis of variance followed by Tukey's test, using SPSS for Windows (15.0 Version). Differences were considered statistically significant when $p < 0.05$.

3. Results

3.1. Histological results

3.1.1. Light microscopic findings

3.1.1.1. Group I (control group). Examination of H&E stained sections of the tongue of the control group showed the tongue section with keratinized papillae covering the underlying connective tissue that contained bundles of skeletal muscle fibers running in different directions (Fig. 1A). A longitudinal section in the skeletal muscle revealed the presence of bundles or fascicles of muscle fibers separated by perimysial connective tissue. The muscle fibers appeared parallel, long, cylindrical and non-branching with acidophilic crossly striated sarcoplasm. The nuclei were multiple, vesicular, elongated and peripheral in position under the sarcolemma. The fibers were connected together by CT endomysium that showed flat nuclei of fibroblasts (Fig. 1B). In transverse sections, the muscle fibers appeared polygonal with acidophilic myofibrils and peripherally located nuclei. The endomysial connective tissue among the muscle fibers showed some blood vessels (Fig. 1C).

3.1.1.2. Group II (animals receiving acrylamide). Longitudinal sections in the skeletal muscle of rats of subgroup IIa (after 20 days of acrylamide administration) showed abnormal wavy course of the muscle fibers with splitting of the myofibers and fragmentation of the sarcoplasm. Moreover, dense pyknotic nuclei and remnants of nuclei

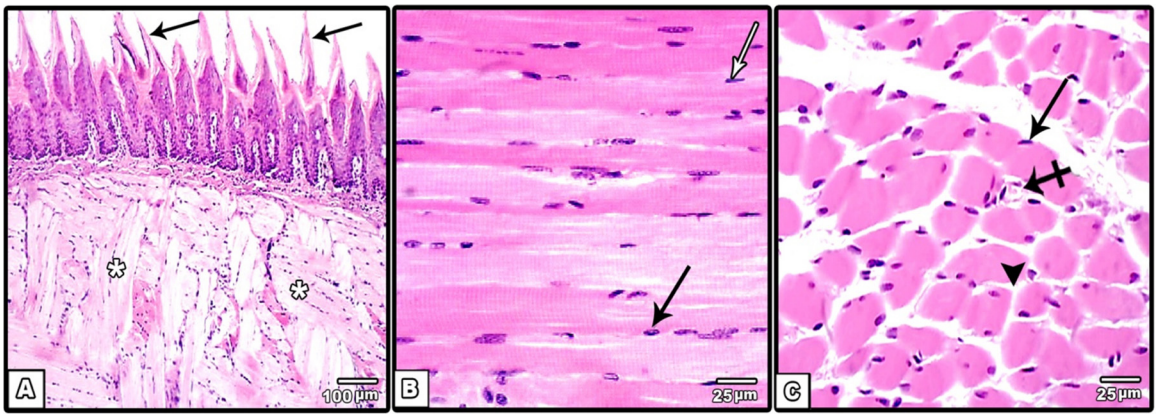

Fig. 1. Photomicrographs of H&E stained sections in the tongue of a control rat (group I). (A) Tongue section with keratinized papillae (arrows) covering the underlying connective tissue that contains normal skeletal muscle fibers (*) running in different directions. (B) A longitudinal section (LS) showing parallel bundles of muscle fibers with elongated vesicular nuclei (black arrow) peripherally located beneath the sarcolemma. Notice the flat nuclei of fibroblasts (white arrow) in the endomysium between the muscle fibers. (C) A transverse section (TS) showing polygonal shaped skeletal muscle fibers with acidophilic cytoplasm and peripherally located nuclei (black arrow). The individual fibers are separated by loose connective tissue endomysium (arrow heads) containing small blood capillaries (crossed arrow).

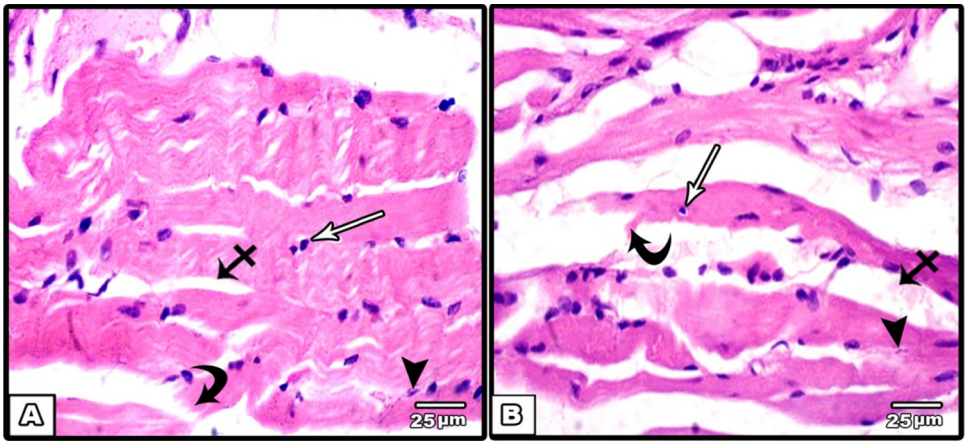

Fig. 2. Photomicrographs of a longitudinal section in the tongue of acrylamide treated rats. (A) Subgroup IIa (acrylamide for 20 days) showing wavy course of the muscle fibers with splitting (crossed arrow) of some fibers. Notice the fragmentation (curved arrow) of the sarcoplasm, the dense pyknotic nuclei (white arrow) and the remnants of nuclei (arrow head). (B) Subgroup IIb (acrylamide for 40 days) showing wide splitting (crossed arrow) of the myofibers. Fragmentation (curved arrow) of the sarcoplasm, the dense pyknotic nuclei (white arrow) and the remnants of nuclei (arrow head) are also present. H&E, Mic. Mag. ×400.

were detected (Fig. 2A). After 40 days of treatment with acrylamide (subgroup IIb) splitting of the myofibers was increased. Fragmentation of the sarcoplasm, dense pyknotic nuclei and remnants of nuclei were also seen (Fig. 2B).

Transverse sections of subgroup IIa showed splitting in some muscle fibers which appeared as a separation in the muscle fiber. In addition, degenerated segments of the muscle fibers, fatty infiltration in between the muscle fibers, and mononuclear cellular infiltration appeared (Fig. 3A). After 40 days of treatment (subgroup IIb), fatty infiltration in between muscle fibers, cellular infiltration and degeneration of the muscle fibers were increased (Fig. 3B).

3.1.1.3. Group III (animals receiving acrylamide and vitamin E). After 20 days of acrylamide and vitamin E administration (subgroup IIIa), H&E stained sections showed mild focal histological changes. Some fibers still have wavy course and splitting of other fibers were also observed. Some mytotic nuclei appeared (Fig. 4A). The histological picture of tongue muscles of the animals treated with acrylamide and vitamin E for 40 days (subgroup IIIb) was more or less similar to control. Parallel muscle fibers with peripherally located nuclei and minute splitting within the fibers were detected (Fig. 4B).

Transverse sections of subgroup IIIa showed centrally located nuclei in some muscle fibers. Degenerated segments of some muscle fibers were occasionally found

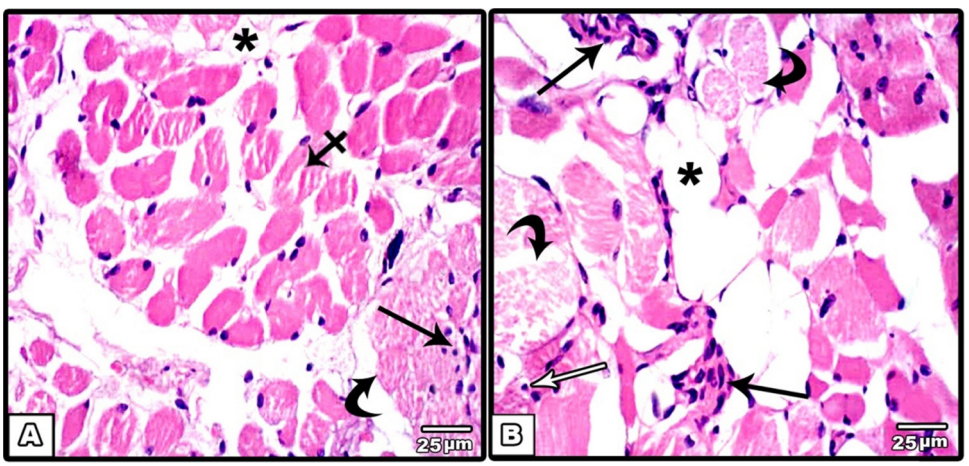

Fig. 3. Photomicrographs of a transverse section in the tongue of acrylamide treated rats. (A) Subgroup IIa showing splitting (crossed arrow), degeneration (curved arrow), cellular infiltration (black arrow) and fatty infiltration (*) of the muscle fibers. (B) Subgroup IIb showing more degeneration (curved arrow), cellular infiltration (black arrow) and fatty infiltration (*) of the muscle fibers. Notice the presence of pyknotic nucleus (white arrow).

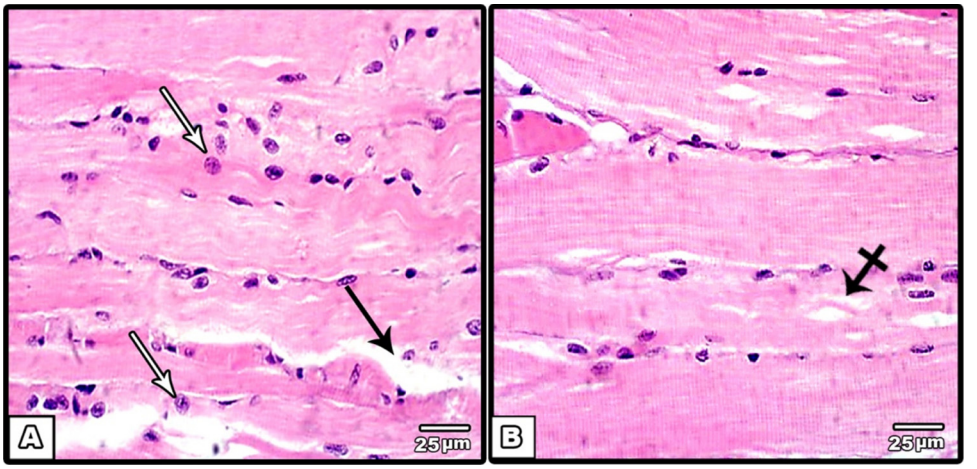

Fig. 4. Photomicrographs of a longitudinal section in the tongue of acrylamide and vitamin E treated rats. (A) Subgroup IIIa (acrylamide and vitamin E for 20 days) showing few wavy course of some fibers. Splitting (arrow) between the muscle fibers is still evident. Notice the presence of mitotic nuclei (white arrow). (B) Subgroup IIIb (acrylamide and vitamin E for 40 days) showing minute splitting (crossed arrow) within the fibers otherwise the myofibrils appear more or less similar to the control group.

(Fig. 5A). After 40 days of treatment (subgroup IIIb) intact muscle fibers with no splitting or fragmentation were seen. Central nucleation of some muscles fibers was clearly evident (Fig. 5B).

3.1.2. Electron microscopic findings

3.1.2.1. Group I (control group). Using the electron microscope, the sarcoplasm appeared filled with myofibrils arranged parallel to the long axis of the myofiber. The myofibrils showed regular arrangement of alternating light (I) and dark (A) bands. A pale narrow region, the H band, could be seen transecting the A band with a dark M line within it. Z line was seen bisecting the light band. Sarcomeres were seen between two successive Z lines. Oval nuclei were seen under the sarcolemma, with their heterochromatin distributed along the inner surface of the nuclear envelope. Mitochondria were seen separating the myofibrils and few mitochondria were also observed at the subsarcolemmal area (Fig. 6).

3.1.2.2. Group II (animals receiving acrylamide). Electron micrographs of longitudinal sections of skeletal muscle fibers of subgroup IIa showed marked aggregation of mitochondria of different size and shape in the subsarcolemmal and intermyofibrillar spaces with degenerated parts of myofibrils (Fig. 7A). Fusion of some mitochondria to form giant ones and vacuolation of others, disruption of Z line and partial loss of myofilaments were observed. Moreover, numerous pinocytotic vesicles were present beneath the plasma membrane and between the myofibrils (Fig. 7B). In subgroup IIb, areas of myofibrillar disarray with loss of sarcomere pattern were observed. Mitochondria were apparently decreased in number. Disorganization and lysis of myofibrils were also detected (Fig. 7C). In addition, small

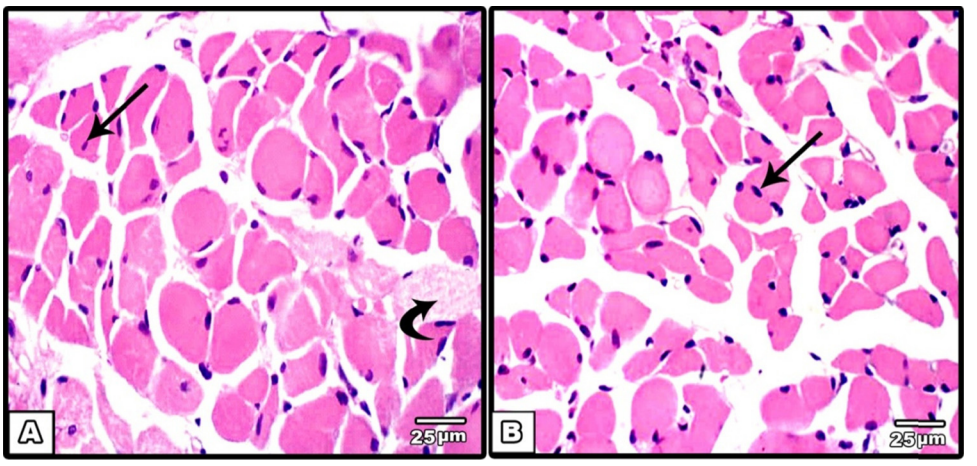

Fig. 5. Photomicrographs of a transverse section in the tongue of acrylamide and vitamin E treated rats. (A) Subgroup IIIa showing polygonal shaped skeletal muscle fibers with acidophilic cytoplasm and peripherally located nuclei. Notice the presence of centrally located nuclei (arrow) in some muscle fibers and degeneration (curved arrow) of other fibers. (B) Subgroup IIIb showing polygonal shaped skeletal muscle fibers with acidophilic cytoplasm and peripherally located nuclei. Central nucleation (arrow) of some muscle fibers is clearly evident.

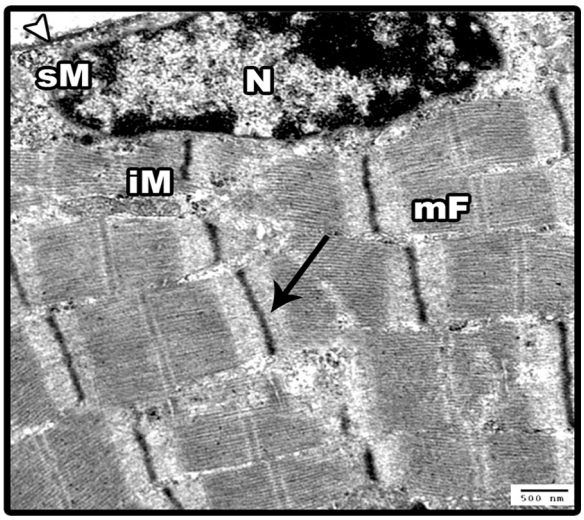

Fig. 6. An electron micrograph of a longitudinal section in the tongue of a control rat (group I) showing parallel arrangement of myofibrils (mF) with light and dark bands. Sarcomeres are seen between two successive Z lines (black arrow). The oval elongated nucleus (N) lies just beneath the sarcolemma (arrow head). Notice the presence of subsarcolemmal mitochondria (sM) and intermyofibrillar mitochondria (iM) (TEM ×5000).

darkly stained degenerated mitochondria with focal disruption and lysis of Z lines were noticed (Fig. 7D).

3.1.2.3. Group III (animals receiving acrylamide and vitamin E). Electron micrographs of longitudinal section of skeletal muscles of subgroup IIIa showed aggregation of mitochondria in the intermyofibrillar space and parallel arrangement of myofibrils with light and dark bands (Fig. 8A). Fused giant mitochondria with clear cristae in the intermyofibrillar space, disruption of Z line and partial loss of myofilaments were also noticed (Fig. 8B).

After 40 days of treatment (subgroup IIIb) large numbers of mitochondria in the subsarcolemmal space and in pairs around the Z line were seen (Fig. 8C). In addition, parallel arrangement of myofibrils with light and dark bands and clear successive Z lines were also observed (Fig. 8D).

3.2. Morphometric and statistical results

In Table 1, the results of the current study showed a statistically significant increase in MDA, pyknotic cells and degenerated muscle fibers in rats exposed to acrylamide for 20 days than the control group in both treated and untreated while, there was statistically significant decrease in total antioxidant capacity. Moreover, statistically significant decrease in MDA, pyknotic cells and degenerated muscle fibers was demonstrated in treated group with vitamin E for 20 days than untreated group with increase in total antioxidant capacity.

A nearly similar results were observed in Table 2 as there was statistically significant increase in MDA, pyknotic cells and degenerated muscle fibers in rats exposed to acrylamide for 40 days than the control with also, decrease in total antioxidant capacity but the reverse was observed in treated group when compared to the untreated group. All markers in the treated 40 days rats showed none statistically significant difference in comparison to the control group.

In Table 3, MDA, pyknotic cells and degenerated muscle fibers showed statistically significant increase in rats received acrylamide for 40 days than those for 20 days and statistically significant decrease in treated 40 days than those treated 20 days rats. Total antioxidant capacity showed a reverse results as there was a statistically significant decrease in untreated 40 days than untreated 20 days rats with increase in vitamin E treated 40 days than vitamin E treated 20 days rats.

4. Discussion

Acrylamide, one of the major environmental public health problems, results from its increased accumulation in the process of cooking food materials [30]. Acrylamide (ACR) toxicity on human and experimental animals was well documented in a series of reports since the Swedish food administration alarm in 2002 [31–33]. Acrylamide is a small organic molecule with very high water solubility. These properties facilitate its rapid absorption and distribution through body [34].

The current study was designed to investigate the possible effects of acrylamide on the skeletal muscle fibers of the tongue of adult male albino rat. Light and electron microscopic examinations of the skeletal muscle fibers were done in addition of estimating the oxidative stress marker MDA and TAC trying to explain the histological changes associated with chronic acrylamide exposure. Moreover, vitamin E was administrated for two different durations for testing its possible protective effects.

In the present study, light microscopic examination of the longitudinal and cross sections of the tongue skeletal muscle fibers as well as electron microscopic examination of the longitudinal sections revealed normal histological structure of tongue skeletal muscles of the adult male albino rats in group I (control group). In subgroup (IIa) in which rats received acrylamide at a dose of 15 mg/kg body weight for 20 days, structural changes appeared. At the level of light microscope, abnormal wavy course of the muscle fibers with splitting of the myofibers and fragmentation of the sarcoplasm were detected. Moreover, dense pyknotic nuclei and remnants of nuclei were observed. It has been mentioned that splitting of muscle fibers is an adaptive response, which occurs when the fiber reaches a critical size at which supply of oxygen and exchange of metabolites are no longer efficient [35].

Transverse sections of subgroup IIa showed splitting in some muscle fibers which appeared as a separation in the muscle fiber. In addition, fatty infiltration in between the muscle fibers, degenerated segments of the muscle fibers and mononuclear cellular infiltration appeared. Fatty and mononuclear cellular infiltrations were also, observed by Almoeiz et al. [36] in their study of the effect of ACR on liver. They found liver damage characterized by the development of cytoplasmic fatty vacuolation and necrosis of the centrilobular hepatocytes with lymphocytic infiltration. Mononuclear cellular infiltration could be explained by the release of certain mediators during degeneration of

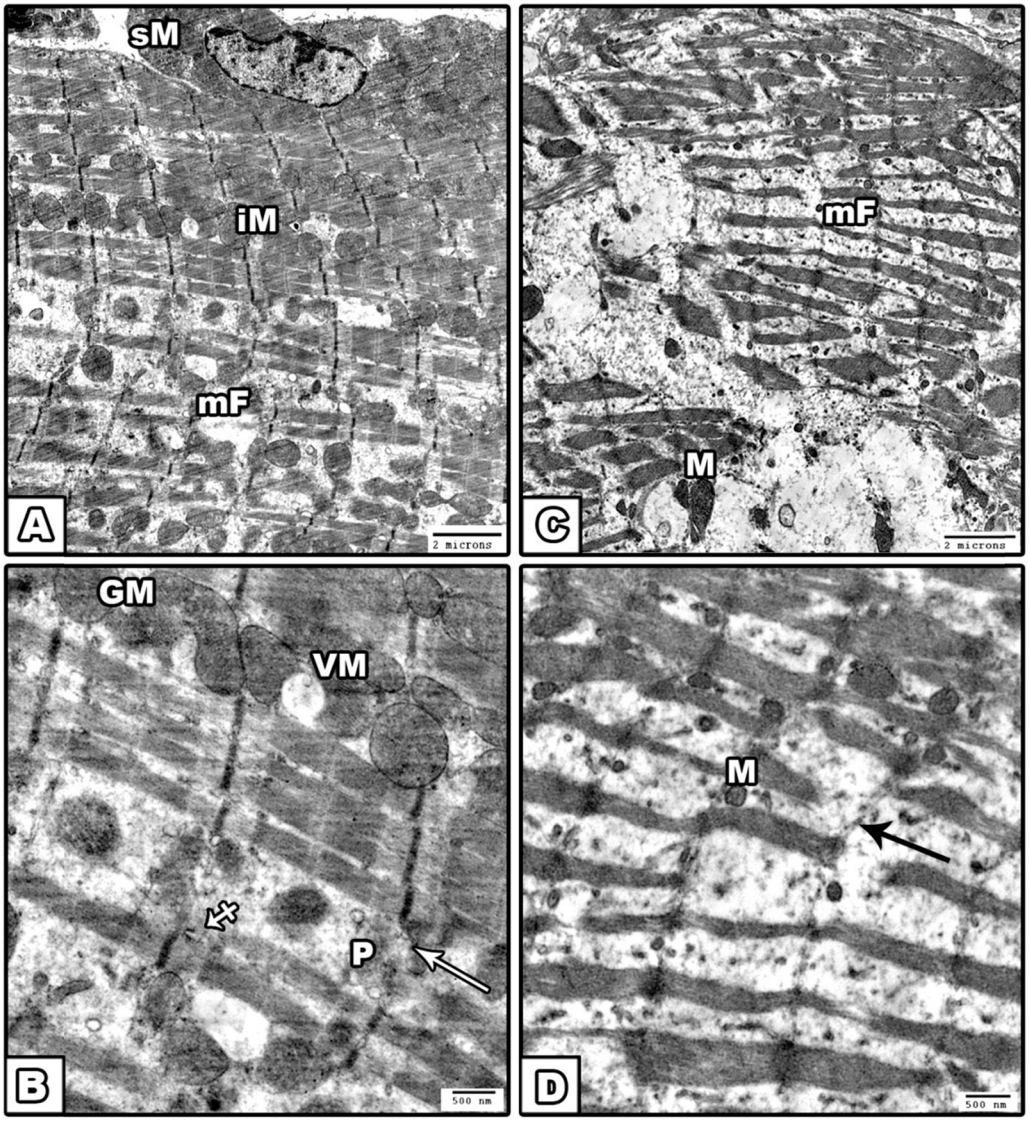

Fig. 7. An electron micrograph of a longitudinal section in the tongue of acrylamide treated rats. (A) Subgroup IIa showing marked aggregation of mitochondria of different size and shape in subsarcolemmal space (sM) and inter myofibrillar space (iM). Notice degeneration of parts of myofibrils (mF) (B) Higher magnification of (A) showing fused giant mitochondria (GM) with clear cristae and vacuolated mitochondria (VM) in the inter myofibrillar space. Disruption of Z line (white arrow), partial loss of myofilaments (crossed arrow) and numerous pinocytotic vesicles (P) are present. (C) Subgroup IIb showing areas of myofibrillar disarray with loss of sarcomere pattern. Apparent decrease in the number of mitochondria (M) and disorganization and lysis of myofibrils (mF) are noticed. (D) Higher magnification of (C) showing small darkly stained degenerated mitochondria (M) and loss of Z line (black arrow).

Table 1
Biochemical and histological parameters in control rats exposed to acrylamide for 20 days (untreated and treated).

	Control	20 days	20 days + vitamin E	ANOVA *p* value
MDA	3.2 ± 0.72	8.6 ± 1.9[a]	5.3 ± 1.2[b,c]	<0.0001
Total antioxidants	0.65 ± 0.19	0.22 ± 0.06[a]	0.39 ± 0.11[b,c]	<0.0001
Degenerated muscle fibers	0.49 ± 0.17	3.2 ± 0.85[a]	1.3 ± 0.42[b,c]	<0.0001
Pyknotic cells	0.35 ± 0.13	1.9 ± 0.56[a]	0.93 ± 0.28[b,c]	<0.0001

p: Probability test used: ANOVA followed by Tukey.
[a] Significance between control group and 20 days group.
[b] Significance between control group and 20 days group + vitamin E.
[c] Significance between 20 days group and 20 days group + vitamin E.

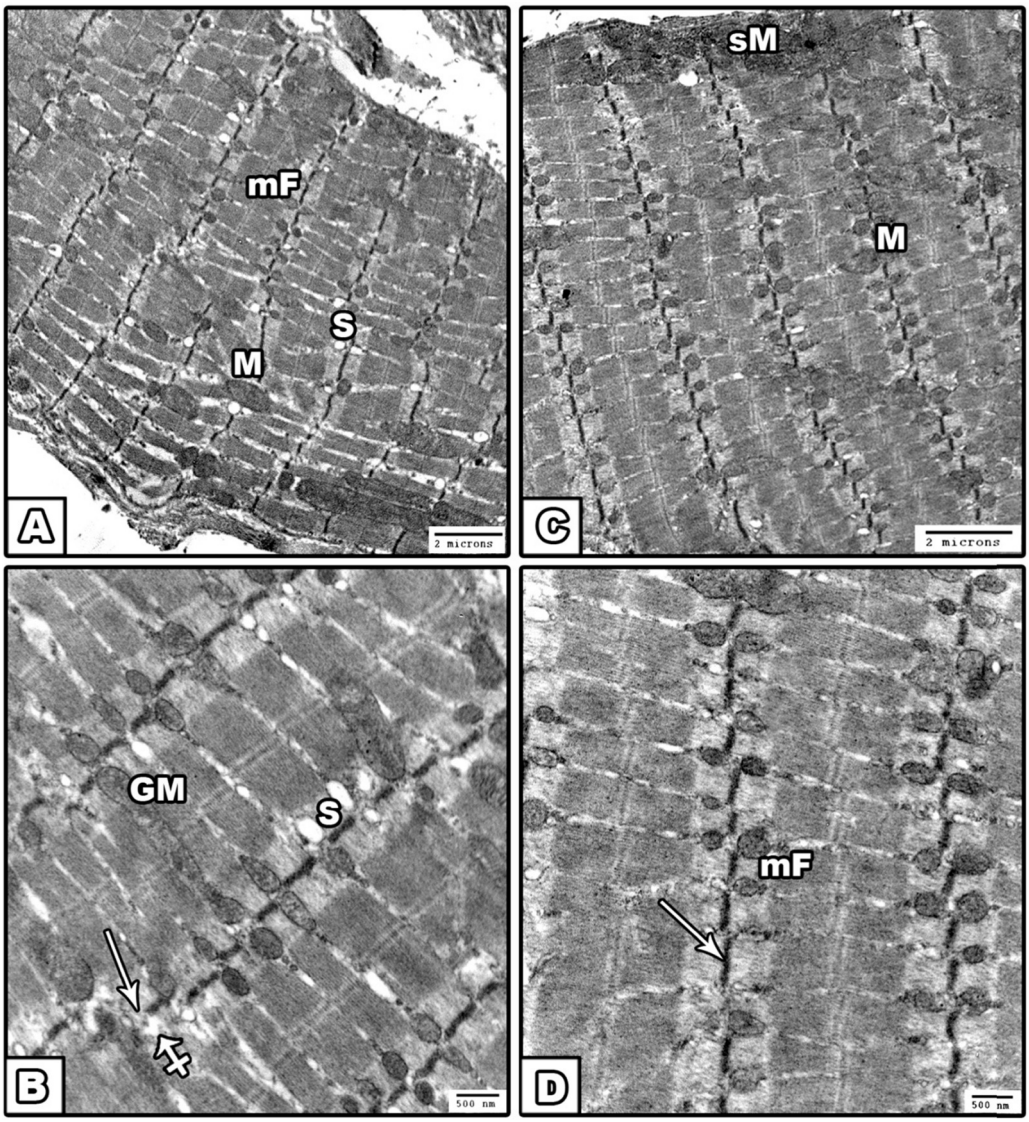

Fig. 8. An electron micrograph of a longitudinal section in the tongue of acrylamide and vitamin E treated rats. (A) Subgroup IIIa showing aggregation of mitochondria (M) in the intermyofibrillar space, parallel arrangement of myofibrils (mF) with light and dark bands. Notice the presence of dilated sarcoplasmic reticulum cisternae (S) (B) Higher magnification of (A) showing fused giant mitochondria (GM) with clear cristae in the inter myofibrillar space, disruption of Z line (white arrow) and partial loss of myofilaments (crossed arrow). Notice the presence of dilated sarcoplasmic reticulum cisternae (S) (C) Subgroup IIIb showing large number of subsarcolemmal mitochondria (sM) and mitochondria (M) in pairs around Z line. (D) Higher magnification of (C) showing parallel arrangement of myofibrils (mF) with light and dark bands and clear successive Z lines (white arrow).

Table 2
Biochemical and histological parameters in control rats exposed to acrylamide for 40 days (untreated and treated).

	Control	40 days	40 days + vitamin E	ANOVA *p* value
MDA	3.2 ± 0.72	12.3 ± 2.3[a]	3.6 ± 1.7[c]	<0.0001
Total antioxidants	0.65 ± 0.19	0.10 ± 0.027[a]	0.59 ± 0.16[c]	<0.0001
Degenerated muscle fibers	0.49 ± 0.17	4.3 ± 0.87[a]	0.57 ± 0.16[c]	<0.0001
Pyknotic cells	0.35 ± 0.13	2.7 ± 0.86[a]	0.38 ± 0.14[c]	<0.0001

p: Probability test used: ANOVA followed by Tukey.
[a] Significance between control group and 20 days group.
[b] Significance between control group and 20 days group + vitamin E.
[c] Significance between 20 days group and 20 days group + vitamin E.

Table 3
Biochemical and histological parameters in rats exposed to acrylamide for 20 and 40 days (untreated and treated).

	20 days	40 days	*p* value	20 days + vitamin E	40 days + vitamin E	*p* value
MDA	8.6 ± 1.9	12.3 ± 2.3	0.006	5.3 ± 1.2	3.6 ± 1.7	0.018
Total antioxidants	0.22 ± 0.06	0.10 ± 0.027	<0.0001	0.39 ± 0.11	0.59 ± 0.16	0.004
Degenerated muscle fibers	3.2 ± 0.85	4.3 ± 0.87	0.01	1.3 ± 0.42	0.57 ± 0.16	<0.0001
Pyknotic cells	1.9 ± 0.56	2.7 ± 0.86	0.02	0.93 ± 0.28	0.38 ± 0.14	<0.0001

p: Probability test used: paired *t*-test.

myocytes which initiate inflammatory reaction and attract inflammatory cells [37].

The previous histological changes were exaggerated with prolongation of the duration of exposure, as regard the fatty infiltration in between the muscle fibers, the mononuclear cellular infiltration and the fragmentation of the muscle fibers. This was confirmed previously by Shinomol et al. [38] as they stated that the toxic effects of ACR in different tissues is a dose dependent which increased by increasing the dose or the duration.

Electron micrographs of skeletal muscle fibers of subgroup IIa showed marked aggregation of mitochondria of different size and shape in the subsarcolemmal and intermyofibrillar spaces with degenerated parts of myofibrils. Fusion of some mitochondria to form giant ones and vacuolation of others, disruption of Z line and partial loss of myofilaments were observed. By 40 days of exposure (subgroup IIb), small darkly stained degenerated mitochondria with focal disruption and lysis of Z lines were noticed. The effect of ACR on mitochondria was described previously by El-Sayyad et al. [39] on retinal cells of rats exposed to ACR with their off springs. It was also stated that morphological mitochondrial abnormalities are most frequently associated with defects of the respiratory chain. Subsarcolemmal accumulations of mitochondria and their proliferation were explained as an attempt by the cell to compensate for the respiratory chain defect [35]. The previous results coincided with our observation and could explain the accumulation of subsarcolemmal mitochondria in this study. Moreover the appearance of giant fused mitochondria in the present work could be explained according to some researchers by the fact that cells are protected from mitochondrial dysfunction by complementation of DNA products in fused mitochondria and thus compensate for defects induced by various kinds of damage [40,41].

The findings of the current study are mostly related to the oxidative stress effect of acrylamide on the skeletal muscle and it coincides with the study of Hori et al. [42] who stated that destruction of muscle fibers with edema and inflammatory cell infiltration are prominent features of oxidative stress effect on skeletal muscle. Their findings were also, observed in our study as there were degenerated segments of the muscle fibers and mononuclear cellular infiltration in subgroup IIa that increased more in subgroup IIb. Similar histological features were observed by Weiss et al. [43]. They noticed that cross-sectional area of myofibers was decreased significantly in association with increased oxidative damage in addition to fatty infiltration, endomysial fibrosis which manifested by increased extracellular matrix between myofibers and these coincides with the result of our study.

Apoptosis which is presented by appearance of dense pyknotic nuclei and remnants of nuclei could be explained also, by the oxidative stress damage effect of acrylamide on skeletal muscle which proved by Sumizawa et al. [44] and Lakshmi et al. [45]. They stated that oxidative stress and apoptosis play an important role in ACR induced toxicity. ACR also, enhanced levels of lipid peroxidative product, protein carbonyl content, and hydroxyl radical, while, it induced apoptosis through alteration of Bcl-2 family protein expression [45].

In addition to the oxidative stress mechanism of acrylamide effect at the cellular levels, acrylamide would likely affect the expression of proteins, enzymes, lipids and nucleic acids [46]. Such effect is enhanced by decreased production of endogenous cellular antioxidant [25] and depletion of cellular antioxidant reduced glutathione (GSH) [38]. Moreover, there are other metabolic effects of acrylamide on the cell function include formation of free radical, alteration of cytoskeleton elements, cell membrane necrosis and mitochondrial destruction [47]. This is in agreement with our study, as there were mitochondrial changes in the form of small darkly stained degenerated mitochondria with focal disruption and lysis of Z lines. DNA damage induced by acrylamide was reported by Guo et al. [48] in the form of central chromatolysis and marked loss of DNA which may be due to acrylamide and its metabolic glycidamide derivative.

In rodents, ACR is rapidly metabolized and excreted predominantly in the urine as metabolites derived from reduced glutathione (GSH) conjugation. The rest is excreted as glycidamide (GA), glyceramide and N-acetyl-S-(3-amino-2-hydroxy-3-oxopropyl) cysteine [38]. This explains its genotoxic effect through its metabolite glycidamide formed via epoxidation by CYP2E1 and leads to the formation of glycidamide-DNA and hemoglobin adducts [49].

In the current study, MDA and TAC were measured in tissue sample homogenizate of skeletal muscle of the tongue trying to find an explanation of the histological changes at light and electron microscopic levels in rats received ACR. There are statistically significant increase in MDA level and decrease in TAC in group IIa and IIb than the control group and group IIb than group IIa. These findings are indicator of oxidative stress in muscles of ACR exposed rats that increased by prolongation of the duration of exposure. These results coincide with Yousef and El-Demerdash [50] and Catalgol et al. [51] who stated that ACR might indirectly stimulate the antioxidant response via glutathione depletion leading to oxidative stress and ROS production either by itself or during metabolization via CYP2E1 [52]. Moreover, Venkataswamy et al. [53] revealed significant decreased in hepatic and cerebral cortex of

brain antioxidant enzymes activity in chick embryos treated with acrylamide with imbalance between oxidant and antioxidant production as there is decreased activity of superoxide dismutase, catalase, glutathione peroxidase and glutathione-S-transferase enzymes responsible for the loss of detoxification of such free radicals. Also, acrylamide induced the depletion of glutathione and ascorbic acid levels in hippocampus region of chick brain [54] and induce hepatocyte oxidative damage [55]. Recently, in 2014 it is reported that oxidative stress has been demonstrated to be a key mechanism in many ACR induced cell injuries and neurodegenerative diseases [56] as well as testicular and epididymal tissues [57].

In the present study, vitamin E was administrated in combination with ACR for two durations (IIIa, 20 and IIIb, 40 days respectively). The histological picture was improved at 20 days (group IIIa) than the non-treated group (IIa) while that of group IIIb (treated group for 40 days) showed picture nearly similar to that of the control. At the level of light microscope, central nucleation of some muscle fibers was clearly evident. This finding comes in accordance with Minamoto et al. [58] as they stated that central nuclei were reported in cases of muscle fiber regeneration. Improvements in the biochemical parameters (decrease in MDA and increase in TAC in treated than non treated group) also, indicate subsidence of oxidative stress.

The choice of vitamin E for prevention of changes in skeletal muscles of the tongue due to exposure of ACR is based on its utilization for the same purpose but on other organs as testes [59] and spinal cord as well as brain [60]. Vitamin E can protect critical cellular structures against damage from both free radicals such as peroxy radical, hydroxyl radical, and super oxide, and from oxidation products such as manoldialdehyde and hydroxynonenal. Thus, the increased free radicals generated by acrylamide exposure in testes might have been scavenged from the testes during recovery period [59]. Vitamin E, as an important antioxidant, plays a role in inhibition of mutagen formation and repair of membranes and DNA [60].

From the present study, it could be concluded that, chronic exposure to acrylamide might lead to skeletal muscle damage in rat tongue which becomes worth with prolonged duration of exposure. Acrylamide induced oxidative stress is the implicated mechanism of such histological changes. This toxic effect of acrylamide could be minimized when vitamin E is given concomitantly with it by its antioxidant effect.

Conflict of interest

We declare that no benefits in any form have been received or will be received from a commercial party related directly or indirectly to the subject of this article. We also declare that we have no conflicts of interest in connection with this paper.

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