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PROTECTIVE EFFECT OF SWIMMING EXERCISE ON ACRYLAMIDE INDUCED DIAPHRAGMATIC MYOPATHY IN MALE ALBINO RATS

By

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Running Title: Protective role of swimming exercise on acrylamide induced muscle myopathy

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ABSTRACT

Background: Acrylamide (ACR) naturally forms during high-temperature cooking of food. Many studies concluded that ACR has neurological, reproductive toxicities, and can produce carcinogenesis. However, the toxic effect of acrylamide on diaphragmatic muscle is not fully investigated .

Objective: To evaluate the effect of acrylamide on diaphragmatic muscle and the effect of swimming exercise on diaphragmatic muscle after exposure to acrylamide.

Materials and methods: Thirty white male albino rats classified into three groups, control, acrylamide, and (swimming & acrylamide) groups. Muscle enzymes were assayed. The diaphragm was weighed, and diaphragmatic muscle contractions were recorded and assessed histologically .

Results: Acrylamide group significantly increased Creatine kinase-MB (CK-MB) and LDH insignificantly while Troponin I and T decreased. Acrylamide increased Malondialdehyde (MDA) and decreased Superoxide dismutase (SOD). Swimming exercise with acrylamide increased LDH, SOD, decreased Troponin I and T and MDA. Acrylamide increased diaphragmatic muscle weight and decreased muscle contraction immediately and after 30 minutes of activity, swimming exercise returned the weight of the muscle near to normal and increased contractions. Histologically, acrylamide group showed degeneration, splitting, fatty and cellular infiltration, dilatation and hemorrhage of the blood vessel, reduction of glycogen and decrease expression of desmin. Swimming exercise improved the histological architecture of the diaphragmatic muscle, increased glycogen content and the expression of desmin .

Conclusion: Acrylamide induced muscle myopathy by oxidative stress, muscle dystrophy, reduction of glycogen content and decreased desmin expression with reduction of diaphragmatic muscle contraction. Swimming exercise ameliorated the effect of acrylamide on diaphragmatic muscle by reversing the previous undesired effects of acrylamide .

Keywords: Acrylamide, Swimming exercise, Diaphragm, Myopathy.

Abbreviations: LDH (lactate dehydrogenase enzyme), CK-MB (Creatine kinase – MB), MDA (Malondialdehyde) & SOD (superoxide dismutase). ELISA (Enzyme-Linked immunosorbent assay).

INTRODUCTION

Acrylamide is a chemical compound (C3H5NO) used in making polyacrylamide and acrylamide copolymers, which are used in many industrial processes, like production of paper, dyes, plastics, and in the treatment of drinking water and wastewater *(Dotson, 2011)*. They are also found in consumer products, like food packaging, and some adhesives. Acrylamide has been found in certain foods that were heated to a temperature above 120 degrees Celsius (248 F), but not in food that heated below this temperature. French fries and potato chips were found to have higher levels of acrylamide in comparison with other foods *(Ubaoji and Orji, 2016)*.

Acrylamide has many health hazards. Acrylamide has been reported as a neurotoxin, these effects are central and peripheral neuropathy causing hallucinations, drowsiness, distal numbness, ataxia, and distal numbness *(Ubaoji and Orji, 2016)*. It is also toxic to the reproductive system *(Favor, 2005)* and carcinogenic. Many studies in rodent's models reported the relationship between acrylamide exposures and several types of cancer *(Arribas-Lorenzo and Morales, 2012)*. The effect of acrylamide on skeletal muscle has been studied by a few researches *(Al-Serwi and Ghoniem, 2015)*.

Exercise is one of the important methods to enhance physical fitness and overall health *(Kylasov and Gavrov, 2011)*. It increases growth, development, weight loss, strength muscle, and heart, and prevent aging. It is one of the important treatments of obesity and diabetes it has many benefits on skeletal muscles include increased glucose transporters, fatty acid oxidation enzymes and antioxidant levels *(Ryan, 2010)*. Exercise leads to the production of reactive oxygen species (ROS) during muscular contraction which results in an increased expression of antioxidant enzymes and increases its production to protect against muscle damage *(Kim et al, 2013)*.

Acrylamide has proven to induce neuropathy *(Motamedshariaty et al., 2014)*, but its effect on the diaphragmatic muscle isn't fully studied .

The aim of the present work was to study the effect of acrylamide on diaphragmatic muscle and the role of swimming exercise on diaphragmatic muscle after exposure to acrylamide.

MATERIALS AND METHODS

Thirty white male albino rats of local strain, weighing 150 ± 20 grams were fed with standard laboratory diet and water "ad libitum" and housed in the animal house at Faculty of Medicine, Menoufia University under normal light/dark cycle and room temperature. The animals were acclimatized to these conditions for 10 days before the experiment. Rats were kept in stainless steel cages (40 x 35 x 35 cm, 5 rats in each cage). We followed the international guidelines for the care and use of laboratory animals, and the study was approved by the institutional ethical committee on the animal experiment of the faculty of medicine, Menoufia University.

The animals were classified into three groups of ten animals each.

Group (1) Control group.

Group (2) Acrylamide group: Acrylamide was given in a dose of (15mg/kg/day dissolved in 0.9% saline solution and were taken orally by gastric tube, for 20 days *(Al-Serwi and Ghoniem, 2015)*. Acrylamide powder was purchased from Sigma Aldrich, Germany.

Group (3) Swimming exercise and acrylamide group: The swimming program (40 min/day) 5 days/ week for 3 weeks. This group received acrylamide in a dose of (15mg/kg/day orally, for 20 days *(Al-Serwi and Ghoniem, 2015)*.

Weights of rats were measured at the start and at the end of the experiment to detect the change in body weight. At the end of the experiment, retro-orbital blood samples were collected to measure muscle enzymes (Creatine kinase (CK-MB), Lactate dehydrogenase (LDH) enzymes, troponin I and troponin T), tissue malondialdehyde (MDA), and superoxide dismutase (SOD). Rats were then sacrificed by cervical decapitation, the diaphragm was excised, weighed, and diaphragmatic muscles contractions were recorded and finally assessed histologically.

Swimming exercise program: Swimming exercise was performed in a rounded plastic water tank (80 cm × 70 cm) at 32 ±5 ?C and a 55 cm water depth. The protocol had two phases: adaptation and training *(Jiang et al., 2014)*. During the adaptive phase, the rats swam 20 min per day for 5 days. The purpose of adaptation was to reduce water-induced stress *(Contarteze et al., 2008)*. After two day rest, the training period began. The swimming program increased progressively from 20 min to 40 min per day for 5 days in 1st week. The swimming programs in 2nd and 3rd weeks were maintained at 40 min per day .

Determination of muscle enzymes: Retro-orbital blood samples were collected and three milliliters of blood was collected in a clean graduated centrifuged tube and left for clotting at room temperature in a water bath for 15 minutes. The supernatant sera were collected in a dry tube and stored at -20 until usage *(Schermer, 1968)*. These samples were used for measurement of LDH, CK-MB, troponin I and troponin T. LDH was measured using calorimetry, both LDH and CK-MB were purchased from (Biosystem, Spain). Troponin I was determined by Enzyme-Linked immunosorbent assay (ELISA) kits (BioMerieux, Inc., USA). Troponin T was determined by ELISA kits (Thermoscientific, Inc., USA) .

Determination of tissue malondialdehyde (MDA) and superoxide dismutase (SOD): The diaphragm was weighed and homogenized by adding 9 times of the volume of 0.9% saline. The 10% homogenate was centrifuged for 10 min (1800 g/min) and the supernatant was used for measurement of MDA & SOD. Malondialdehyde is a marker of lipid peroxidation was determined using thiobarbituric acid reaction *(Draper et al., 1993)*. Superoxide dismutase was measured using calorimetry method. MDA & SOD were purchased from (Biodiagnostic Company, Egypt).

Recording of direct diaphragmatic muscle contraction using oscillograph: Rats were sacrificed by cervical decapitation. The skin over the thorax was opened, along the right border of the sternum. The frontal part of the right thoracic wall was removed. The mediastinum behind the sternum was incised and a cut was made just above the frontal insertion of the diaphragm, the frontal part of the left thoracic wall was then removed and the phrenic nerve was seen quite distinctly after left lung was removed. The abdominal muscles were

cut along the costal margin and the last rib held with a pair of forceps. The diaphragm was now cut out and weighed. The strip was cut out beyond the tendinous part and put in aerated solution was 7.4 pH and warmed by heated water jacket maintained at 37-38 °C (Kreb's solution). The diaphragm cut into two half (partly used for recording contraction and part for histological assessment). The contraction of diaphragmatic muscle was recorded using oscillograph (Harvard apparatus, USA). The stimulation was carried out by supramaximal electric shock at a rate 2/sec with 50 volts strength for 30 minutes *(El-Kotb et al., 2014)*.

Histopathology study: The diaphragm of rats of different groups was dissected and fixed in 10% neutral buffered formalin then dehydrated in ascending grades of alcohol. Specimens were cleared and embedded in paraffin. After deparaffinized, 3-5 microns thick tissue sections were cut by sliding microtome and were subjected to the following stains; Haematoxylin and eosin stain (H&E) and Periodic Acid Schiff (PAS). Immunohistochemistry of Desmin (D33): Desmin (D33)(Cell Marque, USA) were also done.

For quantitative assessment, the degenerative muscle fibers were counted. The mean area percent was measured for the optical density for PAS reaction in diaphragm tissues and anti-Desmin immune histochemical reaction. These measures were done using (Image J 1.47v, National Institute of Health, Bethesda, MD, USA) at Anatomy and Embryology Department, Faculty of Medicine, Menoufia University). For each group of rats, slides from studied animals were examined and five fields were analyzed.

Statistical analysis:

We used SPSS version 16 in our research. The data were analyzed as mean ± standard error of the mean (S.E.M). The significance of difference between means was determined using ANOVA (analysis of variance) followed by post hoc Tukey's test. $p<0.05$ was significant.

RESULTS

Changes in body weight (g): Swimming and acrylamide group showed a significant decrease (39±2.62, $p<0.0001$) when compared to control and acrylamide groups (69±2.69 and 63.7±4.1 respectively). There was no significant change between control and acrylamide groups (Fig. 1).

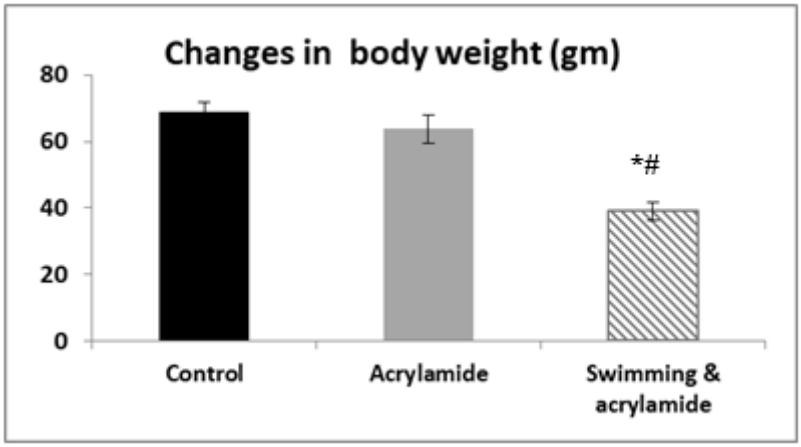

Figure (1): Changes in body weight (g) in control, acrylamide and swimming & acrylamide groups.

Values were measured as mean ± S.E.M. * Significant when compared to control group. #Significant when compared to acrylamide group.

Measurement of LDH (U/l) and CK-MB (ng/ml) enzymes: LDH in Swimming & acrylamide group showed significant increase 1836.8±87.3U/l when compared to control and acrylamide groups (1527.8±31.2 U/l, $p<0.01$ and 1619±62.6 U/l, $p<0.05$ respectively). CK-MB in acrylamide group showed significant increase 128.7±2.55 ng/ml when compared to control and (Swimming & acrylamide) groups (103.9±707ng/ml, $p<0.01$ and 98.2±3.13ng/ml, $p<0.001$ respectively – Fig 2a & b).

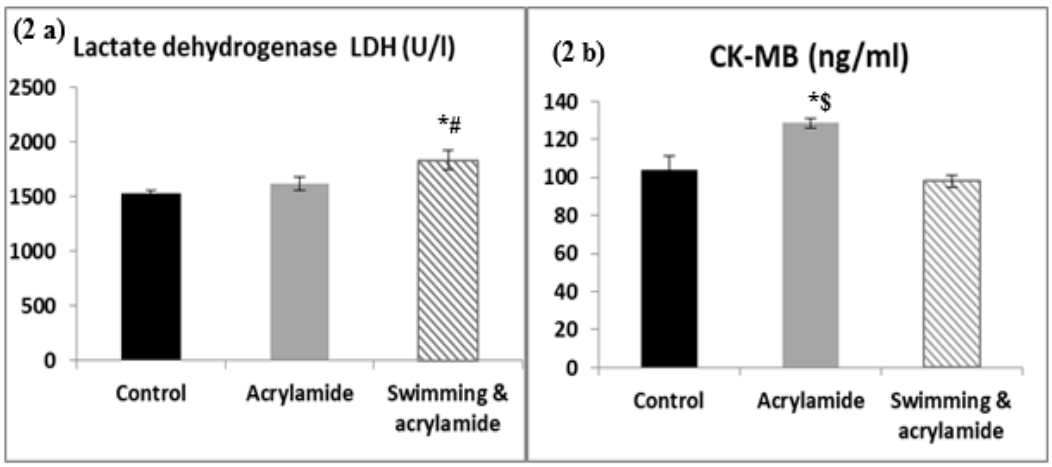

Figure (2a & b): LDH (U/l) and CK-MB (ng/ml) in control, acrylamide and (swimming & acrylamide) groups.

Values were measured as mean ± S.E.M. * Significant when compared to control group. # Significant when compared to acrylamide group. $ Significant when compared to (swimming &acrylamide) group.

Troponin I (ng/ml) and troponin T (pg/ml): Troponin I in acrylamide, and swimming & acrylamide groups showed significant decrease (0.63±0.017 ng/ml and 0.65±0.016 ng/ml, respectively, $p<0.0001$) when compared to control group 0.86±0.027 ng/ml. Troponin T, swimming & acrylamide group showed significant decrease 67.03±2.33 pg/ml, $p<0.05$ when compared to control group

74.2±1.69 pg/ml. There were no significant difference between acrylamide and control, and no significant difference between acrylamide and swimming & acrylamide groups (Table 1).

Table (1): Troponin I (ng/ml) and troponin T (pg/ml) in control, acrylamide and (swimming & acrylamide) groups.

Groups / Parameters	Control	Acrylamide	Swimming and acrylamide	ANOVA
Troponin I (ng/ml)	**0.86±0.027**	**0.63±0.017***	**0.65±0.016***	**P<0.0001**
Troponin T (pg/ml)	**74.2±1.69**	**69.14±1.29**	**67.03±2.33***	**P<0.05**

Values were measured as mean ± S.E.M. * Significant when compared to control group.

MDA (nmol/g) and SOD (µ/g): MDA, acrylamide group showed a significant increase (48.1±5.45 nmol/g, p<0.0001) when compared to control group (5.7±1.64 nmol/g). Swimming exercise with acrylamide administration significantly decreased MDA (28.08±5.8 nmol/g, p<0.05) when compared to acrylamide group, and significant increase (p<0.01) when compared to control group (Fig 3a). SOD, acrylamide group showed significant decrease (0.038 ±0.0037µ/g, p<0.001) when compared to control group (0.062± 0.0045 µ/g). Swimming exercise with acrylamide administration significantly increased SOD (0.047±0.0025 µ/g, p<0.01) when compared to acrylamide group and a significant decrease (p<0.05) when compared to control group (Fig. 3b).

a **b**

Figure (3a & b): MDA (nmol/g) and SOD (µ/g) in control, acrylamide and (swimming & acrylamide) groups

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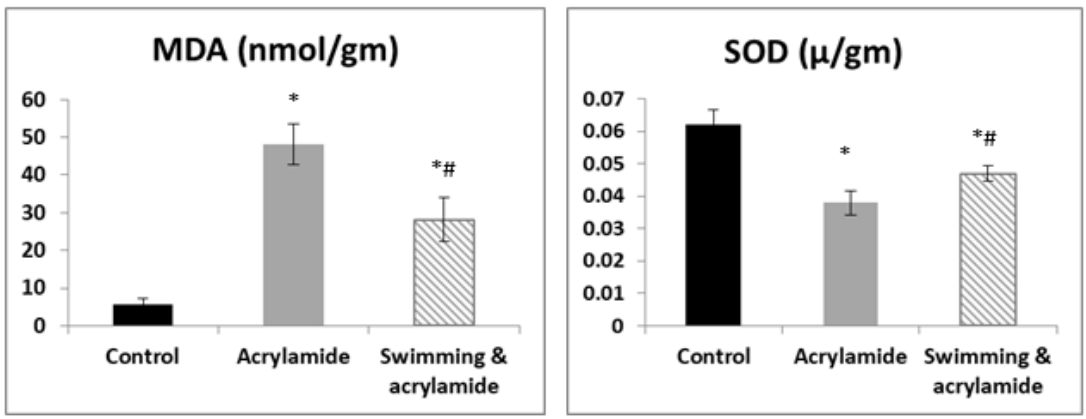
Values were measured as mean ± S.E.M. * Significant when compared to control group. # Significant when compared to acrylamide group.



Diaphragmatic muscle weight and direct muscle contraction: Acrylamide group showed significant increase in muscle weight (0.87±0.03) when compared to control (0.74±0.018, p<0.01) and (swimming &acrylamide) (0.76±0.02, p<0.05) groups. Regarding muscle contraction (immediate and after 30 minutes of activity), acrylamide group showed a significant decrease when compared to control and swimming &acrylamide groups, p<0.0001. Swimming &acrylamide group showed a significant increase when compared to acrylamide group, p<0.0001 (Table 3 & Fig 4).

Table (2): Diaphragmatic muscle weight/g and direct muscle contraction/g tension (immediate and after 30 minutes of activity).

Groups / Parameters	Control	Acrylamide	Swimming and acrylamide	ANOVA
Muscle weight/g	**0.74±0.018**	**0.87±0.03***	**0.76±0.02**	***P<0.01** **$P<0.05**
Muscle contraction/g tension (Immediate)	**3.8±0.07**	**1.8±0.15***	**3.1±0.08*#**	***#$P<0.0001**
Muscle contraction/g tension (after 30 of minutes)	**2.96±0.059**	**1.07±0.05***	**1.7±0.06*#**	***#$P<0.0001**

Values were measured as mean ± S.E.M. * Significant when compared to control group. #

Significant when compared to acrylamide group. $ Significant when compared to swimming &acrylamide group.

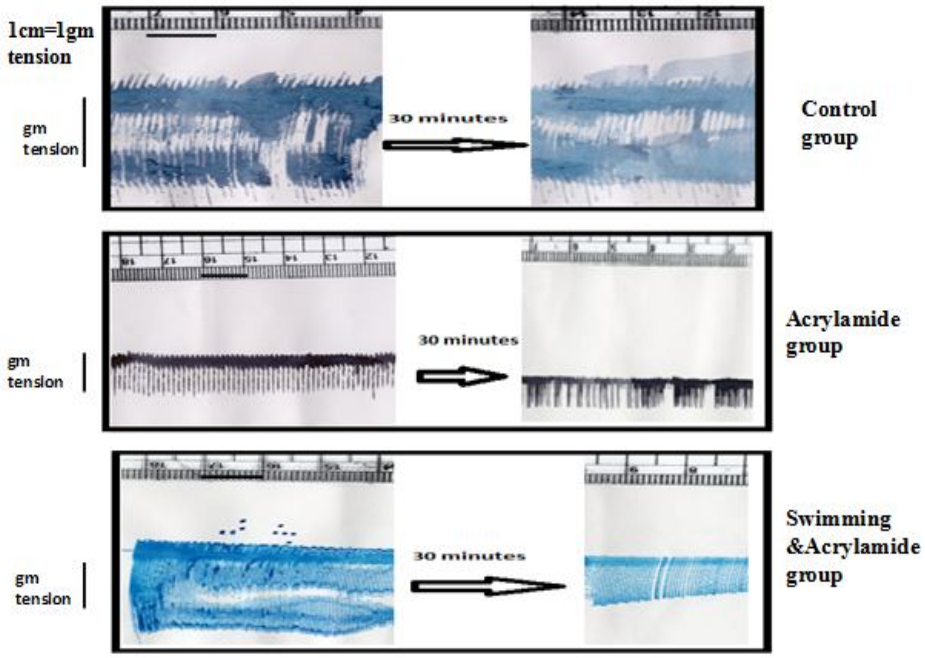


Figure (4): Direct diaphragmatic muscle contraction/g tension (immediate and after 30 minutes of activity) in control, acrylamide and swimming & acrylamide groups.

Histopathological result :

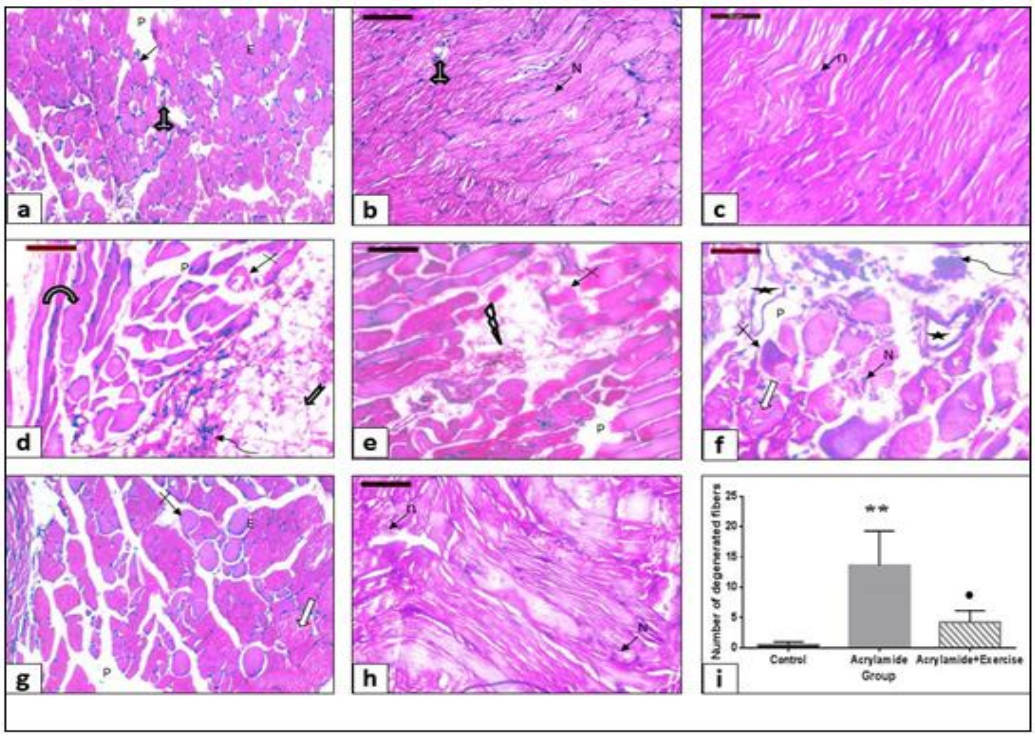


Figure (5): H&E staining of rat diaphragm of different groups. In control groups a transverse section showing polygonal-shaped muscle fibers with acidophilic cytoplasm and peripherally located nuclei (n). The individual fibers are separated by loose connective tissue endomysium (E) containing small blood capillaries (tailed arrow). The diaphragm made up of small bundles which are surrounded by a perimysium connective tissue (p). b, and c. A longitudinal section showing parallel bundles of muscle fibers with elongated vesicular nuclei (n) peripherally located beneath the sarcolemma. In acrylamide group, degenerated fibers (crossed arrow), wavy course of the muscle fibers (curved arrow) with the splitting of some fibers (white arrow), wide endomysium (E) were observed. Fatty infiltration (notched arrow), cellular infiltration (curved arrow), dense pyknotic nuclei (N), dilation of blood vessels (star), and hemorrhage from others were noticed (corrugated line) (d-f). swimming exercise with acrylamide showed significant improvement of histological architecture of the diaphragmatic sections with almost normal muscle fibers, however, some muscle fibers were degenerated (crossed arrow) and some nuclei showed mitosis (N) (g,h). ** Significant ($p<0.001$) compared with control, ●Significant ($p<0.05$) compared with acrylamide group (i). Scale bar = 100 µm (a, b, d, e, and g) and 50 µm (c, f, and h).

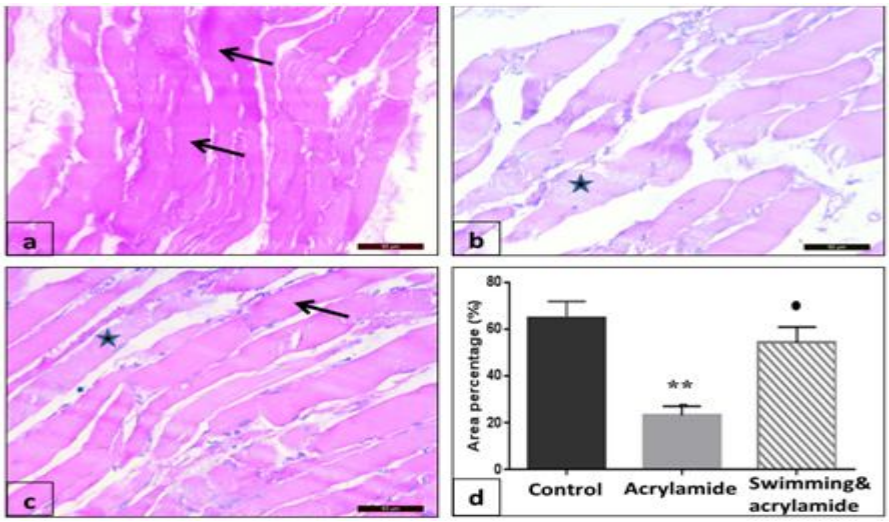


Figure (6): Representative PAS staining of rat diaphragm of different groups. In control group, strong PAS +ve reaction in the sarcoplasm of diaphragm muscle fibers (arrows) was detected (a). Acrylamide treated group showed weak +ve reaction in the sarcoplasm of muscle fibers (stars) (b). Swimming exercise with acrylamide showed areas with moderate +ve reaction (arrow) and areas with weak +ve reaction (star) (c). Periodic acid-Schiff (PAS)-stained sections showing glycogen reduction in acrylamide group compared to the control (a), which increased in (swimming & acrylamide) group (d). ** Significant ($p<0.001$) compared with control, ● significant ($p<0.05$) compared with acrylamide group (i). Scale bar = 50 µm.

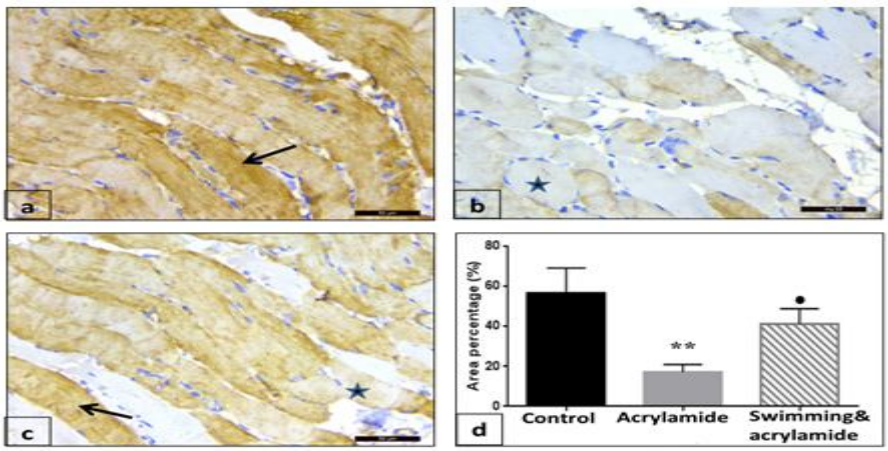


Figure (7): Representative Desmin staining of rat diaphragm of different groups. In control group, strong desmin reaction (arrow) was detected (a). Acrylamide treated group showed weak desmin reaction (stars) (b). Swimming exercise with acrylamide showed areas with strong reaction (arrow) and areas with weak reaction (star) (c).Swimming exercise significantly up-regulated the acrylamide-induced decrease in expression of desmin in the muscle fibers of the diaphragm. (d) ** Significant ($p<0.001$) compared with control, ● significant ($p<0.05$) compared with acrylamide group (i). Scale bar = 50 µm

DISCUSSION

Acrylamide has many health hazards. The toxic effect of acrylamide on the body has been investigated by many types of research *(Dotson, 2011* and *Yang et al., 2016)*. However, the toxic effect of acrylamide on diaphragmatic muscle is not fully investigated. In the present research, the toxic effect of acrylamide on the diaphragm and the possible protective effect of swimming exercise on diaphragmatic muscle after exposure to acrylamide were investigated. (Swimming & acrylamide) group showed a significant decrease in body weight when compared to control and acrylamide groups. This result was in agreement with previous study *(Metz et al., 2016)*, that reported that exercise decreases fat deposition in male and female rats and decrease body weight.

Acrylamide group had a non-significant increase in LDH when compared to control group. This result was in agreement with *Rajeh and Al-Dhaheri (2017)* who reported that acrylamide did not affect LDH activity even in different doses. That indicates that sub-acute levels of ACR exposure over a short period of time do not cause the release of detectable limits of LDH in the serum of the patients. While swimming exercise with acrylamide significantly increased LDH, this result may be due to muscle fatigue induced by exercise and accumulation of lactate and thereby increase expression of LDH as LDH has a preventive effect on muscle fatigue *(Wan et al., 2017)* .

Creatine kinase-MB enzyme was detected as a marker of tissue damage as in myocardial infarction and muscle diseases *(Brancaccio et al., 2007* and *Baird et al., 2012)*. CK-MB was significantly increased in acrylamide group when compared to other groups. This result may be due to the damaging effect of acrylamide on diaphragmatic muscle, this hypothesis was supported by our histological result and the oxidative imbalance. Exercise following Acrylamide administration significantly decreases CK-MB. This result may be due to the improvement effect of swimming exercise on diaphragmatic muscle fiber as detected by our histological result. *(Lee et al., 2012)* reported that swimming exercise has a protective effect by preventing the destruction of skeletal muscle fiber, and improved muscle atrophy caused by diabetes.

Troponin I and troponin T are sensitive markers of myocardial damage. Other non-cardiac diseases detected troponin levels like pulmonary embolism, sepsis, subarachnoid hemorrhage, renal failure, and inflammatory muscle conditions e.g. polymyositis and dermatomyositis *(Dhir and Jiang, 2013)*. Acrylamide and (swimming & acrylamide) groups showed a decrease in troponin I and T when compared to control group these result may be due to acrylamide and swimming exercise have no effect on the cardiac muscle and don’t cause damage of cardiac muscle but, the decrease than control group is not fully understood .

Our results detected oxidative imbalance in the acrylamide group as the elevated MDA and reduced SOD which may be involved in cellular damage, this was in agreement with *Sadek (2012)* who concluded that acrylamide caused significant decrease of SOD, glutathione level and catalase activity and increases in

MDA due to the oxidative stress caused by acrylamide on membrane of rat's stomach, kidney, and liver. Exercise following Acrylamide administration significantly decreases MDA and elevates SOD, These results were in agreement with *Qi et al. (2016)* who concluded that swimming exercise decreased the damage in the skeletal muscle cells by inhibition of oxidative stress in skeletal muscles and the decrease in lipid deposition.

The weight of the diaphragm was significantly increased in acrylamide group when compared to control and (swimming & acrylamide) groups, most probably due to cellular and fatty infiltrations as well as dilated blood vessels and hemorrhage detected by light microscope. Other researches showed a decrease in skeletal muscle weight and muscle atrophy with acrylamide administration *(EFSA, 2015)*.

Our results revealed that the muscle contraction either the immediate or after 30 minutes showed a significant decrease in the acrylamide group when compared to control and (swimming & acrylamide) group, this is may be due to the myopathy occurred with acrylamide administration and detected by light microscopy as cellular and fatty infiltrations as well as dilated blood vessels and hemorrhage these results were in agreement with *Biltz and Meyer (2017)* who concluded that adipocytes accumulation within the skeletal muscle is a common manifestation of muscle myopathies and decreased functional capacity & contractility may be also due to the oxidative stress caused by elevated MDA and reduced SOD these results were in agreement with *Choi et al. (2016)* who stated that increased oxidative stress associated with muscular dystrophy, decreased glycogen deposits in the diaphragm of rats detected by PAS stain may be a cause of decreased contractility this was in agreement with *? rtenblad et al. (2013)* who concluded that intramyofibrillar glycogen has a key role during repeated contractions by counteracting contractile impairments caused by defective release of sarcoplasmic reticulum Ca^{+2}, also our results revealed a decrease expression of desmin in the muscle fibers of the diaphragm which affect muscle contraction this was consistent with the results of *Palmisano et al. (2015)* who detected the central role of desmin in both mechanical stress transmission and transduction in muscle. They have demonstrated that desmin is specifically involved in myofibrillar alignment, nuclear integration within the myofibrillar matrix, mechanical response to high stress, and stress-mediated signaling in muscle. These results demonstrate that the Z-disk, and its desmin cytoskeleton, plays a central role in both muscle signal transduction and force transmission in muscle fibers.

Our results revealed significant increase in muscle contraction in swimming & acrylamide group when compared to acrylamide group this was in agreement with *Steinbacher and Eckl (2015)* who concluded that the repetitive muscle contractions during endurance training lead to a variety of responses including phenotypic and physiological responses, which cause activation of mitochondrial biogenesis, transformation of fiber type and angiogenesis.

Light microscopic examination of the diaphragm revealed the normal histological structure of diaphragmatic muscles of the adult male albino rats in control group. Rats that received acrylamide their diaphragmatic sections showed degenerated muscle fibers with dense pyknotic nuclei and wavy appearance with the splitting of some myofibers. The pathologists have been described these changes to be muscle myopathy *(Joyce et al. (2012). Al- Serwi and Ghoneim (2015)* observed a similar finding in rat tongue exposed to acrylamide .

The splitting of muscle fibers is an adaptive response, which occurs as a result of an inefficient supply of oxygen and exchange of metabolites and increases reactive oxygen species as malondialdehyde as detected by our result. *Soliman et al. (2017)* also suggested that nuclear migration play a part in the pathogenesis of muscle fiber splitting. Degenerated muscle fibers could be explained by the overproduction of reactive oxygen species (ROS) and oxidative stress *(Abou zaid et al., 2017)*.

Cellular and fatty infiltrations, as well as dilated blood vessels and hemorrhage from others, were noticed in acrylamide group. The same results were detected by *Gedik et al. (2017)* in their study of the effect of acrylamide on the liver. *Chen et al. (2018)* explained the cellular infiltration by degeneration of myocytes which lead to the release of certain mediators that initiate inflammatory reaction attracting inflammatory cells. These findings are mostly due to the oxidative stress effect of acrylamide on the diaphragm and it coincides with *Choi et al. (2016)* who stated that increased oxidative stress has been associated with muscular dystrophy.

The detection of glycogen deposits, visualized on PAS-stained sections, was reduced in amount in the diaphragm of rats with acrylamide. The same results reported by *Kovac et al. (2015)*. Reduced glycogen may be due to redirecting the cell metabolism to more intensive acrylamide biotransformation by mobilizing more of glutathione and increasing the activity of glutathione-S-transferase (GST) *(Veenapani et al., 2010)*. This also might be as a result of break-down of glycogen into glucose molecules since *Rawi et al. (2012)* found a strong correlation between an increase of serum glucose level in rats and acrylamide administration.

Our results revealed that acrylamide-induced decrease in expression of desmin in the muscle fibers of the diaphragm. This is in agreement with *Sakai et al. (2009)* who stated that acrylamide as an inhibitor of the intermediate filaments (vimentin and desmin) significantly decreased the contractions induced by acetylcholine (ACh) of the rat isolated bronchial smooth muscle. Moreover, decreased desmin reaction reported in this study in accordance with *Capetanaki et al. (2015)* who said that desmin deficiency cause defects in membranes and membranous organelles resulting in skeletal and cardiac myopathies in mice and humans. The desmin filament system is critical for force transmission and connects Z-discs longitudinally and laterally .

Interestingly, the treadmill exercise significantly ameliorated most of the

above mentioned histological and immunohistochemical changes. These results were in agreement with *Nalbandian et al. (2013)* who reported histological improvement in uphill exercised valosin-containing protein (R155H/+) mice.

Inconsistent with these results *Jensen et al. (2011)* said that exercise increases the glycogen storage capacity in skeletal muscles; they added muscle glycogen is important for survival during acute emergencies as substrate for "fight or flight" reactions Also, *Hearris et al. (2018)* reported that endurance training increases glycogen content in muscles .

In confirmation, *Paulsen et al. (2009)* concluded that both desmin protein content and the mean power generated by the muscle were increased after exercise training; they added a reinforced desmin cytoskeleton may be necessary for the muscle to increase the force generation.

CONCLUSION

Acrylamide induced muscle myopathy by increasing CK-MB, MDA & degeneration of muscle fibers and decreasing SOD, glycogen content, expression of desmin and diaphragmatic muscle contraction. Swimming exercise with acrylamide ameliorated the effect of acrylamide on diaphragmatic muscle and had a protective effect against muscle myopathy induced by acrylamide by reversing the previous undesired effect of acrylamide.

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REFERENCES

1. **Abou zaid O, EL-sonbaty S, Ezz W and Barakat M (2017):** Ameliorative effect of selenium nanoparticles and ferulic acid on acrylamide-induced neurotoxicity in rats. Annals of medical and biomedical sciences, 3 (2): 35-45.
2. **Al-Serwi RH and Ghoneim FM (2015):** The impact of vitamin E against acrylamide induced toxicity on skeletal muscles of adult male albino rat tongue: Light and electron microscopic study. Journal of Microscopy and Ultrastructure, 3: 137–147.
3. **Arribas-Lorenzo G and Morales FJ (2012):** Recent insights in Acrylamide as carcinogen in foodstuffs. Adv. Mol. Toxicol., 6:163–193
4. **Baird MF, Graham SM, Baker JS and Bickerstaff GF (2012):** Creatine-Kinase- and Exercise-Related Muscle Damage Implications for Muscle Performance and Recovery. Journal of Nutrition and Metabolism, 1- 13.
5. **Biltz NK and Meyer GA (2017):** A novel method for the quantification of fatty infiltration in skeletal muscle. skeletal muscle, 7:1.
6. **Brancaccio P, Maffulli N and Limongelli FM (2007):** Creatine kinase monitoring in sport medicine. British Medical Bulletin, 81-82(1):209–230.
7. **Capetanaki Y, Papathanasiou S, Diokmetzidou A, Vatsellas G and Tsikitis M (2015):** Desmin related disease: a matter of cell survival failure. Curr Opin Cell Biol., 32:113-120.
8. **Chen L, Deng H, Cui H, Fang J, Zuo Z, Deng J, Li Y, Wang X and Zhao L (2018):** Inflammatory responses and inflammation-associated diseases in organs. Oncotarget, 9(6):7204.
9. **Choi MH, Ow JR, Yang ND and Taneja R (2016):** Oxidative Stress-Mediated Skeletal Muscle Degeneration: Molecules, Mechanisms, and Therapies. Oxidative Medicine and Cellular Longevity, 1-13.
10. **Contarteze RV, Manchado FB, Gobatto CA, and De Mello MA (2008):** Stress

biomarkers in rats submitted to swimming and treadmill running exercises. Comp. Biochem. Physiol. Part A. Mol. Integr. Physiol., 151: 415–422.

11. **Dhir T and Jiang N (2013):** Misleading elevation of troponin T caused by polymyositis. International Journal of biomedical science: IJBS., 9(2):107.
12. **Dotson GS (2011):** NIOSH Skin Notation (Sk) Profile: Acrylamide [CAS No. 79-06-1]. US Department of Health and Human Services. Public Health Service, Centre for Disease Control and Prevention, National Institute for Occupational Safety and Health DHHS (NIOSH) Publication, 2011(2011-139):1-3.
13. **Draper HH, Squires EJ, Mahmoodi H, Wu J, Agarwal S and Hadley MA (1993):** Comparative evaluation of thiobarbituric acid methods for the determination of malondialdehydes in biological materials.Free Rad Biol Med., 15: 353-363.
14. **EFSA Panel on Contaminants in the Food Chain (CONTAM) (2015):** Scientific opinion on acrylamide in food. EFSA Journal, 13(6):4104.
15. **El-Kotb S, Naguib Y and El-domiaty H (2014):** Effect of experimental hyperthyroidism on the maximum acute exercise tolerance and neuromuscular performance in rats. Menoufia Medical Journal, 27:78–84.
16. **Favor J (2005):** Shelby Transmitted mutational events induced in mouse germ cells following acrylamide or glycidamide exposure Mutat Res., 580 (2005): 21–30.
17. **Gedik S, Erdemli ME, Gul M, Yigitcan B, Gozukara Bag H, Aksungur Z and Altinoz E (2017):** Hepatoprotective effects of crocin on biochemical and histopathological alterations following acrylamide-induced liver injury in Wistar rats. Biomed Pharmacother., 95: 764-770.
18. **Hearris M, Hammond K, Fell J and Morton J (2018):** Regulation of muscle glycogen metabolism during exercise: implications for endurance performance and training adaptations. Nutrients, 10(3):298.
19. **Jensen J, Rustad PI, Kolnes AJ and Lai YC (2011):** The role of skeletal muscle glycogen breakdown for regulation of insulin sensitivity by exercise. Front Physiol., 2(112):1-11.
20. **Jiang P, Dang R, Li H , Zhang L, Zhu W, Xue Y, and Tang M (2014):** The Impacts of Swimming Exercise on Hippocampal Expression of Neurotrophic Factors in Rats Exposed to Chronic Unpredictable Mild Stress, Evidence-Based Complementary and Alternative Medicine, 1- 8.
21. **Joyce NC, Oskarsson B and Jin LW (2012):** Muscle biopsy evaluation in neuromuscular disorders. Physical Medicine and Rehabilitation Clinics, 23(3):609-31.
22. **Kim JH, Kwak HB, Thompson LV and Lawler JM (2013):** Contribution of oxidative stress to pathology in diaphragm and limb muscles with Duchenne muscular dystrophy. J Muscle Res Cell Motil., 34:1–13.
23. **Kovac R, Rajkovic V, Koledin I and Matavulj M (2015):** Acrylamide alters glycogen content and enzyme activities in the liver of juvenile rat. Acta Histochem., 117(8):712-717.
24. **Kylasov A and Gavrov S (2011):** Diversity of Sport: non-destructive evaluation. Paris: UNESCO: Encyclopedia of Life Support Systems, 462–491.
25. **Lee Y, Kim J-H, Hong Y, Lee S-R, Chang K-R and Hong Y (2012):** Prophylactic effects of swimming exercise on autophagy-induced muscle atrophy in diabetic rats. Lab Anim Res., 28(3): 171–179.
26. **Metz L, Gerbaix M, Masgrau A, Guillet C, Walrand S, Boisseau N, Boirie Y and Courteix D (2016):** Nutritional and exercise interventions variably affect estrogen receptor expression in the adipose tissue of male rats. Nutrition Research, 36(3):280-9.
27. **Motamedshariaty VS, Farzad SA, Nassiri-As M and Hosseinzadeh H (2014):** Effects of rutin on acrylamide-induced neurotoxicity. Daru, 22(1): 22-27.
28. **Nalbandian A, Nguyen C, Katheria V, Llewellyn KJ, Badadani M, Caiozzo V and Kimonis VE (2013):** Exercise training reverses skeletal muscle atrophy in an

experimental model of VCP disease. PLoS One, 8(10):e76187.

29. **? rtenblad N, Westerblad H and Nielsen J (2013):** Muscle glycogen stores and fatigue. J Physiol., 591(18):4405-4413.

30. **Palmisano MG, Bremner SN, Hornberger TA, Meyer GA, Domenighetti AA, Shah SB, Kiss B, Kellermayer M, Ryan AF and Lieber RL (2015):** Skeletal muscle intermediate filaments form a stress-transmitting and stress-signaling network. J Cell Sci., 128: 219-224.

31. **Paulsen G, Lauritzen F, Bayer ML, Kalhovde JM, Ugelstad I, Owe SG, Hallen J, Bergersen LH and Raastad T (2009):** Subcellular movement and expression of HSP27, αB-crystallin, and HSP70 after two bouts of eccentric exercise in humans. Journal of applied physiology, 107(2):570-82.

32. **Qi J, Yang B, Ren C, Fu J and Zhang J(2016):** Swimming Exercise Alleviated Insulin Resistance by Regulating Tripartite Motif Family Protein 72 Expression and AKT Signal Pathway in Sprague-Dawley Rats Fed with High-Fat Diet. J Diabetes Res., 1-9.

33. **Rajeh NA and Al-Dhaheri NM (2017):** Antioxidant effect of vitamin E and 5-aminosalicylic acid on acrylamide induced kidney injury in rats. Saudi medical journal, 38(2):132.

34. **Rawi SM, Marie MAS, Fahmy SR and ElAbied SA (2012):** Hazardous effects of acrylamide on immature male and female rats. Afr.J.Pharm.Pharmacol., 6:1367–86.

35. **Ryan AS (2010):** Exercise in aging its important role in mortality, obesity and insulin resistance. Aging health, 6(5):551-63.

36. **Sadek M (2012):** Antioxidant and immunostimulant effect of carica papaya linn. Aqueous extract in acrylamide intoxicated rats. Acta Inform Med., 20 (3):180-185.

37. **Sakai H, Kozutsumi Y, Goto K, Chiba Y and Misawa M (2009):** Functional importance of the cytoskeletal protein in acetylcholine-induced contraction of rat bronchial smooth muscle. J Smooth Muscle Res., 45 (2-3):97-108.

38. **Schermer S (1968):** Rats haemopiotic system in: Blood morphology of laboratory animals. 1st ed. Pbl. Davis. A Co. Philadelphia, 10: 112.

39. **Soliman ME, Atteia SE, Kefafy MA, Ali AF and Radwan EM (2017):** Histological study on the effect of rosuvastatin (Crestor) on the skeletal muscle of adult male albino rats and the possible protective effect of coenzyme Q10. Menoufia Medical Journal, 1; 30(4):1117.

40. **Steinbacher P and Eckl P (2015):** Impact of Oxidative Stress on Exercising Skeletal Muscle. Biomolecules, 5(2): 356-377.

41. **Ubaoji KI and Orji VU (2016):** A review on acrylamide in foods: Sources and implications to health. Mgbakoigba: Journal of African Studies, 6(1).

42. **Veenapani SN, Meena Bai M, Uma A, Venkatasubbaiah K, Rao KJ and Thyagaraju K (2010):** Glutathione S transferase protein, nucleic acid, chromatin, cell nuclei and structural variation analysis of erythrocyte, bone marrow cell and hepatocytes of rats under the influence of acrylamide. Bioscan, 5:477–481.

43. **Wan JJ, Qin Z, Wang PY, Sun Y and Liu X (2017):** Muscle fatigue: general understanding and treatment. Experimental & molecular medicine, 49(10):e384.

44. **Yang S, Cao C, Chen S, Hu L, Bao W, Shi H, Zhao X and Sun C (2016):** Serum metabolomics analysis of quercetin against acrylamide-induced toxicity in rats. Journal of agricultural and food chemistry, 64(48):9237-9245.

التأثيرالوقائي لممارسة السباحة على الاعتلال العضلي الغشائي الناجم عن الأكريلاميد عند ذكور الجرذان

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خلفيـــة البحـــث: يتكــون الأكريلاميــد اثنــاء طهــى الطعــام فــي درجــات الحــرارة العاليــة. وخلصــت العديــد مــن الدراســات إلــى أن الاكريلاميــد لــه ســمية عصــبية وتناســلية ، ويمكــن ان يــؤدى إلــى مــرض الســرطان. ومــع ذلــك ، فــإن التــأثير الســام للأكريلاميــد على العضلات الغشائية لم يتم التحقق منه.

الهــدف مــن البحــث: تقيــيم تــأثير الأكريلاميــد علــى العضــلات الغشــائية وتــأثير ممارســة السباحة على العضلات الغشائية بعد التعرض للأكريلاميد.

مــواد وطــرق البحــث: إســتخدم فــى هــذا البحــث ثلاثــون مــن ذكــورالجرذان البيضــاء، وقســمت الــى ثــلاث مجموعات:مجموعــة الــتحكم، و مجموعــة الأكريلاميــد، ومجموعــة الســباحة مــع الأكريلاميــد. وقــد تــم قيــاس إنزيمــات العضــلات ووزن عضــلة الحجاب الحاجزوتسجيل إنقباضها ودراسة أانسجتها.

النتــائج: زادت نســبة الكريــاتين كينازوالمالوندالدهيــد بينمــا انخفــض التروبــونين اى وتــى وسوبراوكســيد ديســموتازفى مجموعــة الاكريلاميــد، بينمــا زاد معــدل انــزيم اللاكتيــت ديهيدروجينازوسوبراوكســيد ديســموتازوانخفض التروبــونين اى وتــى و المالوندالدهيــد فــى مجموعــة الســباحة مــع الاكريلاميــد. زاد الأكريلاميــد مــن وزن العضــلات الغشــائية وانخفــض تقلــص العضــلات علــى الفــور وبعــد 30 دقيقــة مــن النشــاط ، كمــا أعــادت ممارســة الســباحة وزن العضــلات وزيــادة الانقباضــات بــالقرب مــن الطبيعــى .نســيجيا: أظهــرت مجموعــة الأكريلاميــد انتكــاس ، وتقســيم ، وتســلل الدهنيــة والخلويــة ، وتوســع ونزيــف الأوعيــة الدمويــة ونقــص معــدل الديســمين والجليكــوجين ، بينمــا أدت ممارســة الســباحة إلــى تحســين البنيــة النســيجية للعضــلة الغشائية وزيادة محتوى الجليكوجين ومعدل الديسمين.

الخلاصـــة: الأكريلاميـــد يســـبب إعـــتلال فـــى العضـــلات الغشـــائية للجـــرذان وذلـــك بســـبب الإجهـــاد التأكســـدي ،وضـــمور فـــى العضـــلات ،و تقليـــل محتـــوى الجليكـــوجين، وتقليـــل معـــدل الدســـمين مـــع تقليـــل إنقباضـــات العضـــلات الغشـــائية، بينمـــا أدت ممارســـة الســـباحة إلـــى تحســـين تـــأثير الأكريلاميـــد علـــى العضـــلات الغشـــائية عـــن طريـــق عكـــس الآثار غير المرغوبة فيها للأكريلاميد.