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Role Of Acrylonitrile Toxicity In Lung Of Albino Male Rats

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Abstract

The aim of this study to investigate the effectiveness of Acrylonitrile (AN) in albino male rats, the rats (n= 130) were randomly and equally into 2 group: 1st group act as control group feeding on rat diet, 2nd group administration daily orally by the stomach tube AN (40mg/ kg BW) for 90 days, AN was prepared by dissolving of 0.8 ml of AN in 100 ml of D.W. (0.8% v/v). In 90 day of study taken the blood samples for serum Alpha fetoprotein (AFP) assay by Elisa technique & lung tissue. Results indicated that AN significantly increased ($P<0.05$) in AFP concentration, pathological changes showed peribronchial lymphoid proliferation with emphysema, interstitial pneumonia with alveoli fibrosis, necrosis and collapse of alveoli septa. In conclusion the AN showed intoxicant and induced pronounced hazardous effects and pathological changes in lung rats.

Keywords: Acrylonitrile, Alveolar macrophage, GC, albino male rats.

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Introduction

Hazardous chemicals have dangerous toxic effects on the environment and human health, moreover producing these chemicals involve the use and handling of other hazardous chemicals are used in the manufacturing process. Harmful effects of chemical pollutants and their metabolites are due to their ability to generate reactive oxygen species (ROS) due to ingestion, inhalation or absorption chemicals and their products[1]

Acrylonitrile (AN) it's a highly chemical poisonous compound with the formula (C3H3N), colorless, volatile liquid in a characteristic odour with boiling point (77.3 Co), onion or garlic - like odor , although commercial samples can be yellow due to impurities, also AN (CH2 = CH − C ≡ N) is soluble in water and miscible with most organic solvents , Technical-grade AN is more than 99% pure, with minor quantities of impurities and stabilizers [2] Acrylonitrile is used mostly to make plastics, acrylic fibers, and synthetic rubber because acrylonitrile evaporates quickly, it is most likely to be found in the air around chemical plants where it is made. AN breaks down quickly in the air, it has been found in small amounts in the water and soil near manufacturing plants and hazardous waste sites [3, 4]

The AN induced pathological changes in rats characterized by stomach degeneration and atrophy in the glandular epithelium with edema and diffuse inflammatory cells infiltration, also appear dilated blood vessels in the submucosal layer, target organs are the hemorrhagic gastrointestinal tract appear hemorrhage , hemorrhagic in the adrenal gland and necrosis, brain and lung edema, the overexposure to the AN characterized by mild jaundice, cyanosis (dark red, blue or purple skin colors in lips, gums or fingernails), irritation with inflammation of eyes and respiratory system including nose and threat [5, 6]

Materials and methods

The present experiment were applied on Lab. Animal (Albino male rats) 130 male rats were involved in this study, the age 8-9 weeks and the weight (200-220) gms, the animals were housed in plastic cages an air-conditioned room with temperature maintained at 25 ±20C, this plastic cages containing hard-wood chip as bedding and bedding was changed continuously to ensure a clean environment. Rats were given food pellets and water ad libitum and all rats were randomized into two group for 90 days:

1- 1st group act as control group feeding on rat diet.
2- 2nd group administration daily orally by the stomach tube AN (40mg/ kg BW) for 90 days.

The dose of AN was 40mg/kg B.W. according to [5] and it was prepared by dissolving of 0.8 ml of AN in 100 ml of D.W. (0.8% v/v).

All animals were sacrified under ethics protocol under slight ether anesthesia for post mortem for Elisa technique and pathological examination at days 90 of the experiment.

Blood collection:

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The fasting blood heart was used to detection of rat alpha fetoprotein (AFP) by using Elisa technique via cardiac puncture technique in the test tube and left 15 minutes to stand and then coagulate in refrigerator at 500 rpm for 15 minute in centrifuge, serum was separated and kept at -20 C^o for analysis, the assay was carried out in the College of Medicine – Al-Nahrain University, by using Rat AFP ELISA kit from (AVIVA systems biology, China).

Pathological Examination:

At days 90 of experiment all animals are sacrified by longitudinal abdominal opening, lung was carefully dissected to record any abnormal in size, color, consistency, adhesion, immediately fixed in 10% neutral buffered formalin throw ascending graded of ethanol (70,80,90 and 100%) 1st run and 100% 2nd run, then xylene after that embedded in paraffin and staining by routine stain (hematoxylin and eosin) [7]

Statistical analysis:

All the grouped data were statistically read by SPSS program, Version 17 software (2010)[8, 9].Testing methods including one way ANOVA for comparisons among groups[10]. P values of less than <0.05 were considered statistical significance, and all data were expressed as means ± standard error (SE). [11, 12]

Results

Detection of Serum rat Alpha Fetoprotein (AFP) by ELISA:

Rat alpha Fetoprotein (AFP) in below table showed significant increase (P<0.05) in 2nd group (40.1 ± 2.9 ng/mL) compared with control group.

Pathological examination:

The macroscopic changes in lung showed Solid consistency with pale areas of voluminous emphysema, gray to white area, in cut section congestion with severe edema as fluid oozes from surface with white froth in the bronchus and other section fill the thoracic cavity and distended alveolar sac (fig. 1). While the microscopic changes showed Peribronchial lymphoid cuffing lymphocytic cells and marked peribronchial lymphoid proliferation with emphysema and congested blood vessels and alveoli collapse (fig. 2). Emphysematous lung characterized by diffuse distention of the alveoli and interstitial thickening (fig. 3). Chronic interstitial pneumonia due to macrophages present in interstitial with alveoli fibrosis, increased cellularity, smooth musculature and focal lymphoid aggregation (fig. 4). Lung tissue showed necrosis and collapse of alveoli septa , all blood vessels congested with interstitial hemorrhage and granuloma (fig. 5), Also the pleura thickening with underlying irregular granuloma with foamy macrophage and apoptosis (fig. 6).

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Table (1) : Serum rat AFP (ng/mL) concentration in male rats.

Parameters Groups	AFP (ng/mL)
Control (1st group)	9.1 ± 1.5[c]
Acrylonitrile (2nd group)	40.1 ± 2.9[a]

Values are expressed as means±SE with different letters are significantly different (P<0.05).

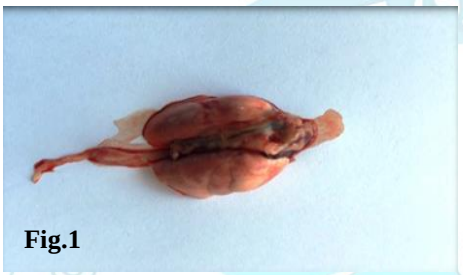


Fig.1

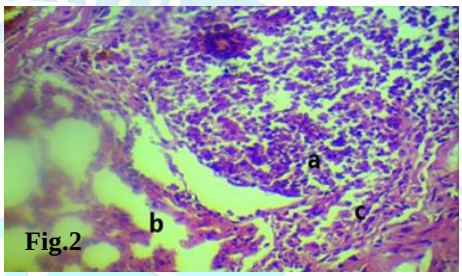


Fig.2

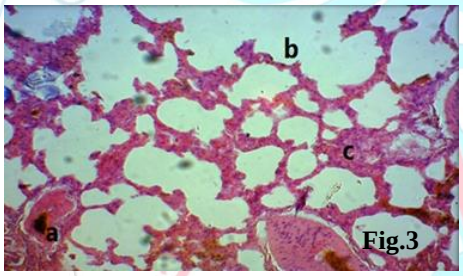
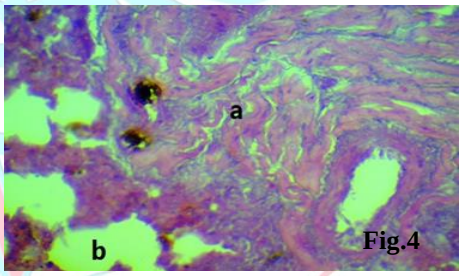


Fig.3



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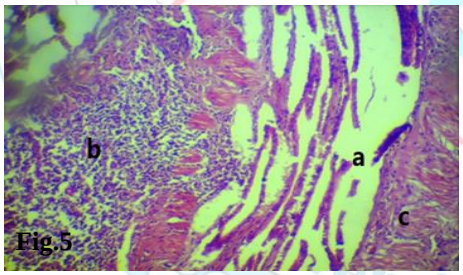

Fig.5

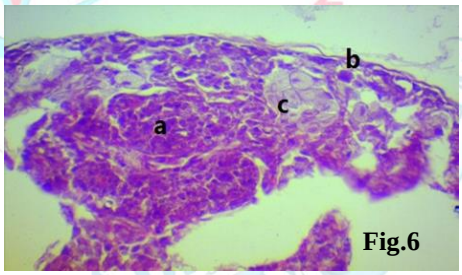

Fig.6

Figure (1) : Grossly appearance of lung in 2nd group at (90) days showed congested with areas of depressed pale color.
Figure (2): Histopathological changes of lung in 2nd group showed a) Peribronchial lymphocytic cuffing cells hyperplasia b) Emphysema alveoli c) Alveoli collapse. (X40 H&E)
Figure (3): Histopathological changes of lung in 2nd group showed a) Moderate hyperemia of alveolar wall with congested vessels b) Emphysema alveoli c) Thickening in interstitial layer.(X40 H&E)
Figure (4): Histopathological changes of lung in 2nd group showed a) Fibrosis and increase thickening of smooth musculature b) Emphysema in alveoli.(X40 H&E)
Figure (5): Histopathological changes of lung in 2nd group showed a) Disruption of alveolar tissue b) Prominent of lymphoid tissue c) Increase thickening smooth musculature.(X20 H&E)
Figure (6): Histopathological changes of lung in 2nd group showed a) granulomatous lesion in pulmonary parenchyma b) Pleura thickening by foamy macrophage c) Apoptotic phenomena.(X20 H&E)

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Discussion :

Serum Alpha Fetoprotein (AFP):

The AFP was thought to be associated chiefly with overt liver cancer in man and animals and most of the results reported here showed that all of the chemical hepatocarcinogens (HCC) tested induced AFP-producing tumors.

The current study showed there was a significant increase of AFP in 2nd group. These results are in agree with [13],stated that the AFP synthesis is reactivated in liver tumors and germinogenesis in a lesser degree after chemical and mechanical liver injuries followed by regeneration such as acute viral hepatitis.

Zhou *et al*., (2010) demonstrated that the high serum concentration of AFP in HCC might be due to the tumor excretion of this protein[14], furthermore some clinical researches indicated that the high serum concentration of AFP is closely related to poor differentiation and biologically malignant characteristics especially due to portal vein invasion of hepatocellular carcinoma (HCC) [15]

Pathological changes:

The 2nd group showed histopathological changes in lung tissue including hyperplasia of bronchial lymphoid, emphysema, interstitial pneumonia, and collapse of alveoli septa, these results are similar to those reported by [16]who observed alteration in histopathology of lung tissue in rats that exposed to dichlorvos. The results are in agree with [17] who showed focal necrotic area and peribronchial aggregation of inflammatory cells in lung tissue of rat exposed to acrylamide for 6 week and some of alveoli contain eosinophilic exudate and emphysema. However, these results are inconsistent with those documented by [18] who that observed no changes in the lung tissue of rats exposed to dichlovos for 4 week. On other hand [19] who reported that there are histopathological alteration in the lung exposed to polyacrylamide and might be due to irritating effects on bronchiolar and alveolar epithelial. The results are in agree with [20] who found that there were respiratory disturbances and pulmonary edema with moderate to marked hyperplasia of clear cells lining the bronchioles after administration single oral dose (46.5mg/kg BW) of AN to male Sprague Dawley rats.

Moreover, the inflammatory cells infiltrating were also appeared mainly MNCs , lymphocytes and neutrophils in 2nd group, these changes may be due to the toxic effect of AN and phagocytic cells (macrophages, dendritic and B lymphocytes) that resulting in increased the susceptibility to toxicity and infection [21]

Conclusion:

Oral administration of AN (40mg/kg BW) induced pronounced hazardous effects in rats, and cause significantly increased in AFP level with pathological changes in lung mostly granulomatous reaction, hyperplasia, pneumonia, necrosis and collapse of alveoli septa.

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