Effective Dose of Vitamin C Against Passive Smoking
Soheir F. Nour 1, Tayseer M. El-Assar 2 and Noha M. Zahran 3

ABSTRACT

Objective: Ascorbic acid (AA), known as vitamin C, plays an important role in the human body. Therefore, this study aimed to estimate the beneficial effects of two levels of (AA) that can protect or cure the lung against the hazards of passive smoking in male mice.

Materials and Methods: The study was done on forty male albino mice (6 weeks age and average body weight 22 ± 3 g). The mice were assigned into eight equal groups; control group, group 2 was exposed to cigarette smoke for 12 weeks, and the other six groups were given low or high doses of vitamin C (0.015 and 0.075 mg/g BW) before, during and after exposure to smoke. Doses were given orally via oro-gastric tube.

Results: High doses of vitamin C had a protective effect when taken before and during exposure to smoke. It reduced the negative effect of smoke as indicated by the levels of TBARS and GSH in plasma and lung tissue, and the improvement of studied histological parameters.

Conclusion: Ascorbic acid had no therapeutic effects after lung tissue damage due to exposure to secondhand smoke. Therefore, it is important to protect the lung from the negative effects of smoking by increasing the daily intake of vitamin C doses to 1 gram, from food rich in vitamin C like fresh fruits and vegetables.

Keywords: Vitamin C, Passive smoking, Lung, Biochemical, Histological examinations

INTRODUCTION

Cigarettes carry serious health risks, which are more prevalent than other tobacco products. About half of cigarette smokers die from tobacco-related disease and lose on average 14 years of life (Doll et al., 2004). Passive smoking causes many of the same diseases as direct smoking including respiratory diseases and lung cancer. Cigarettes produce an aerosol containing over 4,000 chemical compounds, including nicotine, carbon monoxide, acrolein, and other harmful substances, over 50 of these are carcinogenic (Seget et al., 2012).

Passive smoking is a mixture of smoke from the burning end of a cigarette, and the smoke exhaled from the lungs of smokers. It is involuntarily inhaled, lingers in the air hours after cigarettes have been extinguished, and can cause a wide range of adverse health effects, including respiratory infections, asthma and cancer (ALA, 2010).

Nonsmokers who are exposed to passive smoking at home or work increase their heart disease risk by 25–30% and their lung cancer risk by 20–30% (CDCP, 2013). Sudden infant death syndrome, ear infections, respiratory infections, and asthma attacks can occur in children who are exposed to passive smoking. Scientific evidence shows no level of exposure to second-hand smoke is safe (CDCP, 2012).

Dietary antioxidants are an important factor in protecting against the damaging effects of oxidative stress in the airways, a characteristic of respiratory diseases (Wood et al., 2005). Oxidative stress caused by reactive oxygen species (ROS), is generated in the lungs due to various exposures, such as air pollution (Kelly, 2005). Antioxidants vitamins including vitamin C, vitamin E, flavonoids and carotenoids are abundantly present in fruits and vegetables, as well as nuts, vegetable oils, cocoa and green tea.

Some authors dispute the hypothesis that antioxidant vitamins could prevent chronic diseases (NCI, 2007), while others maintain such a possibility is unproved and misguided from the beginning (Huy et al., 2008).

Although certain levels of antioxidant vitamins in the diet are required for good health, there is considerable doubt as to whether antioxidant-rich foods or supplements have anti-disease activity; and if they are actually beneficial, it is unknown which antioxidant(s) are needed from the diet and in what amounts beyond typical dietary intake (Woodside et al., 2005).

It was found that vitamin C reduces the levels of histamine in the blood that increases asthma. Vitamin C in the reduced form, in the lower respiratory tract and airway surface fluids, and has an important role in protecting the lung due to its anti-free radical properties (Yang et al., 1999) (Hemila, 2004) and may help to prevent chronic obstructive pulmonary disease (Berthon & Wood, 2015).

Previous studies differed in identifying the effective dose of vitamin C on the respiratory system, and the results are conflicted on the effect of vitamin C as a protector for the respiratory tract especially the lung.

Therefore, the aim of this study was to determine the effective dose of vitamin C on the lung against cigarette

DOI: 10.21608/ASEJAIQISAE.2020.106475

1Department of Home Economics, Faculty of Agriculture, Alexandria University (Egypt.)
2Agriculture Research Center (Egypt.)
3Histology Department, Faculty of Medicine, Alexandria University (Egypt.)

Received, April 2, 2020, Accepted, June 30, 2020.
smoke exposure, and to evaluate its preventive and/or cure effect using some biochemical parameters and histological tests.

**MATERIALS AND METHODS**

**Materials:** Ascorbic acid was purchased from El-Gomhoreya Co., Egypt. All reagents used in the study were purchased from Bio-diagnostic Co., Egypt and Sigma Chemical Co., Germany.

Two doses of vitamin C were used and defined as high dose (0.075 mg/g body weight) and low dose (0.015 mg/g body weight). Concerning the animal body weight was about 22±3 g.

**Experimental animals:** Forty male Albino mice aged 6 weeks and weighed (22 ± 3 g) at the beginning of the experiment. They were purchased from the Animal House, Department of Home Economics, Faculty of Agriculture, Alexandria University. The animals received a balanced diet and water *ad libitum* throughout the study. They were maintained under standard housing conditions and housed 5 per cage, and kept for two weeks for acclimatization period before start of the experiment (Childs et al., 2002). The experimental procedure followed the rules of research ethics approved by the Research Ethics Committee, Faculty of Agriculture, Alexandria University. Biochemical analysis were carried out in the Central Laboratory, Institute of Graduate studied and Research, Alexandria University. Histological samples were prepared and examined at Histology Laboratory, Faculty of Medicine, Alexandria University.

The basal diet contents are shown in table (1) and table (2).

### Table 1. % Content of basal diet

| Diet Ingredient                                      | %    |
|------------------------------------------------------|------|
| Dextrin                                              | 43.65|
| Casein- Vitamin Free                                 | 21   |
| Sucrose                                              | 15   |
| RP Mineral Mix #10 (adds 1.29% fiber)                 | 5    |
| Corn Oil                                             | 10   |
| Powdered Cellulose                                   | 3    |
| RP Vitamin Mix (adds 1.94% sucrose)                  | 2    |
| Choline Chloride                                     | 0.2  |
| DL-Methionine                                        | 0.15 |

Protein 18.3%, Fat 22.1%, Carbohydrates 59.6%

### Table 2. Minerals and Vitamins mixture composition

| Minerals                  | Vitamins                      |
|---------------------------|-------------------------------|
| Calcium %                 | Vitamin A, IU/g               | 22.1 |
| Phosphorus %              | Vitamin D, IU/g               | 2.2  |
| Potassium %               | Vitamin E, IU/kg              | 50.1 |
| Magnesium %               | Vitamin K, ppm                | 10.4 |
| Sodium %                  | Thiamin, ppm                  | 20.7 |
| Chloride%                 | Riboflavin, ppm               | 20.7 |
| Fluorine, ppm             | Niacin, ppm                   | 90   |
| Iron, ppm                 | Pantothenic acid, ppm         | 56   |
| Zinc, ppm                 | Folic acid, ppm               | 4.2  |
| Manganese, ppm            | Pyridoxine, ppm               | 16.5 |
| Copper, ppm               | Biotin, ppm                   | 0.4  |
| Cobalt, ppm               | Vitamin B12, mcg/kg           | 24   |
| Iodine, ppm               | Choline chloride, ppm         | 1.40 |
| Chromium, ppm             | Ascorbic Acid, ppm            | 0.0  |
| Molybdenum, ppm           |                               |      |
| Selenium, ppm             |                               |      |
Inhalation system was based on the study of (Candon et al., 1995) for using two connected glass boxes, one used as a burning box and the other as an exposure box. The animals were exposed one time a day (2 cigarette per exposure period, 5 days a week). Vitamin C solution was freshly prepared and given orally via oro-gastric tube.

Lung damage were assessed using some indicators such as the lung/body relative weight, oxidative stress indices in blood and lung tissues, as well as investigation of lung histology.

Experimental design: Eight groups were studied for 14 weeks, each had 5 mice (Table 3):

**Group 1 – Negative control (CONT):** served as control kept free from smoke exposure.

**Group 2- Positive control (SMOK):** exposed to smoke for 12 weeks without given vitamin C.

**Group 3-HCBS:** given high doses of vitamin C for two weeks, then exposed to smoke until the end of the experiment.

**Group 4-LCBS:** Mice were treated as group 3 but the dose of vitamin C was low.

**Group 5-HCDS:** Mice were given high doses of vitamin C during exposure to smoke. **Group 6-LCDS:** Mice were treated as group 5 but the dose of vitamin C was low.

**Group 7-HCAS:** Mice were exposed to smoke for 12 weeks, then given high doses of vitamin C only for two weeks after stopping exposure to smoke.

**Group 8-LCAS:** Mice were treated as group 7 but the dose of vitamin C was low.

**Biochemical tests:** Blood samples were collected from abdominal aorta during scarification in tubes containing heparin as anti-coagulant, and then they were placed immediately on ice packs. Plasma was obtained by centrifugation of samples at 4000 RPM (rotation per minute) for 20 minutes, and was separated and stored at -80°C until used for analyses. Stored plasma samples were analyzed for reduced glutathione (GSH) and thiobarbituric acid reactive substances (TBARS) using assay kits from Bio-diagnostic Co. Egypt. Fresh blood samples were immediately sent in ice box to the laboratory for determine its content of vitamin C using commercial kits from Bio-diagnostic Co. Egypt. Lungs were immediately removed, washed, weighted and stored at -80°C until used for analyses. Tissues were minced and homogenized using sucrose solution prepared in concentration (85.57 g sucrose / liter of distilled water), the homogenate was centrifuged at 10,000 xg for 20 min (Youssef, 2012). The resultant supernatant of the tissue was used for measuring (GSH) and (TBARS).

**Histological Analysis:** The chest was opened and the lungs were washed and weight to calculate their relative weight as mg/g of body weight. The lungs were dissected and fixed in 10% formol saline, and processed to get 6 µm thick paraffin sections. These sections were stained with hematoxylin and eosin stain (H and E) for light microscopic examination (Drury& Wallington, 1980).

**Statistical Analysis:**

Data were analyzed according to Steel study (Steel& Torrie, 1981). Statistical significance of the difference in values of control and treated animals was calculated by F test with 5% significance level. Then the data were statistically tested by using Dancan’s Multiple Range Test (SAS, 1986).

### Table 3. The groups’ treatments during the experiment period (14 weeks)

| Groups | Week 1 | Week 2 | Week 3 | Week 4 | Week 5 | Week 6 | Week 7 | Week 8 | Week 9 | Week 10 | Week 11 | Week 12 | Week 13 | Week 14 |
|--------|--------|--------|--------|--------|--------|--------|--------|--------|--------|---------|---------|---------|---------|---------|
| 1      | -      | -      | -      | -      | -      | -      | -      | -      | -      | -       | -       | -       | -       | -       |
| 2      | -      | S      | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       | S       | S       |
| 3      | H      | H      | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       | S       | S       |
| 4      | L      | L      | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       | S       | S       |
| 5      | -      | -      | S+H    | S+H    | S+H    | S+H    | S+H    | S+H    | S+H    | S+H     | S+H     | S+H     | S+H     | S+H     |
| 6      | -      | -      | S+L    | S+L    | S+L    | S+L    | S+L    | S+L    | S+L    | S+L     | S+L     | S+L     | S+L     | S+L     |
| 7      | S      | S      | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       | S       | H       |
| 8      | S      | S      | S      | S      | S      | S      | S      | S      | S      | S       | S       | S       | S       | L       | L       |

G= groups    S= exposure to Smoke    H= high dose    L= low dose
At the end of experimental period (14 weeks), all animals were anesthetized by light ether and sacrificed.
RESULTS AND DISCUSSION

Relative Lung / body weight: As shown from Table (4), there were no significant differences in the relative lung/body weight of all studied groups compared to the control except group (SMOK) which exposed to smoke and did not given vitamin C. Exposure to smoke increased the relative lung weight by (205%). Therefore, it can be concluded that treatment with vitamin C prevent the lung from the risk of increasing its relative weight as a sign of illness.

Vitamin C levels in plasma: Table (5) illustrates the effect of exposure to cigarette smoke on levels of plasma vitamin C of the different mice groups. There are significant differences between the different groups except groups 3 and 5. A significant decrease was found in group 2 exposed to smoke (2 SMOK) compared to control by 31.6%. The vitamin C increased by 453.7% and 242.7% in the HC and LC groups respectively.

Previous findings suggest that exposure to smoking in groups 3 and 4 has significantly reduced vitamin C levels in plasma compared to its level in groups 3 * and 4 *. Although cessation of smoking in groups 7 and 8, the effects of smoke led to lower levels of vitamin C compared to groups 3 * and 4 *.

Oxidative stress: In the current study, assessment of oxidant status of plasma and homogenates lung tissue revealed significant elevation by of TBARS in group 2 (SMOK) compared to control group. However, administration of vitamin C with low and high doses decreased the level of TBARS compared to the smoke exposure group but the high dose was more effective, which mean that the administration of vitamin C before and during exposure to smoke can protect against oxidative stress damage (Table 6).

Table 4. Effect of vitamin C and exposure to smoke on relative lung / body weight

| Groups | CONT | SMOK | HCBS | LCBS | HCDS | LCDS | HCAS | LCAS |
|--------|------|------|------|------|------|------|------|------|
| Mean   | 0.7753a | 2.301b | 0.760a | 0.716a | 0.779a | 0.876a | 0.873a | 0.748a |
| S.D.   | 0.028 | 0.26 | 0.056 | 0.045 | 0.029 | 0.077 | 0.027 | 0.085 |

Different superscript letters were significantly different, P<0.05

Table 5. Effect of exposure to cigarette smoke on vitamin C levels in plasma

| Group | HC | LC | CONT | SMOK | HCBS | LCBS | HCDS | LCDS | HCAS | LCAS |
|-------|----|----|------|------|------|------|------|------|------|------|
| Vitamin C(µg/L) | 743.6a | 460.3c | 134.3b | 91.8d | 352.8d | 160.5e | 356.9d | 194.4f | 638.2b | 258.1c |
| S.D. | 1.70 | 1.4 | 2.58 | 3.5 | 2.8 | 9.8e | 4.7 | 2.5 | 3.6 | 3.4 |
| % change | +453.7 | +242.7 | - ----- | - 31.6 | +162.7 | +19.8 | +165.7 | +44.8 | +375.2 | +92.2 |

Different superscript letters were significantly different, P<0.05
HC=High vitamin C dose,  LC=Low vitamin C dose.
*3 HC: The highest levels of vit.c in plasma in HCBS group after the first two weeks of taking vit.c.
*4 LC: The highest levels of vit.c in plasma in LCBS group after the first two weeks of taking vit.c.

Table 6. Effect of vitamin C and smoke exposure on TBARS in plasma (nmol/ml) and lung tissue (nmol/g)

| Group | CONT | SMOK | HCBS | LCBS | HCDS | LCDS | HCAS | LCAS |
|-------|------|------|------|------|------|------|------|------|
| TBARS in plasma | Mean | 1.90b | 6.53a | 2.26d | 3.63c | 2.60d | 3.76c | 3.34c | 4.64b |
| S.D. | 0.20 | 0.69 | 0.71 | 0.33 | 0.64 | 0.27 | 0.32 | 0.69 |
| % change | ------ | 243.7 | 18.9 | 91.1 | 36.8 | 97.9 | 75.8 | 114.2 |

TBARS in lung

| Mean | 0.76f | 4.03a | 0.86f | 1.44d | 0.91f | 2.02c | 1.18c | 2.31b |
| S.D. | 0.001 | 0.14 | 0.016 | 0.101 | 0.033 | 0.031 | 0.068 | 0.049 |
| % change | ------ | 430.3 | 13.2 | 89.5 | 19.7 | 165.8 | 55.3 | 203.9 |

Values with different superscript letters were significantly different, P<0.05
Table 7. Effect of vitamin C and smoke exposure on GSH in plasma (µmol/ml) and lung tissue (µmol/g)

| Group | CONT | SMOK | HCBS | LCBS | HCDS | LCDS | HCAS | LCAS |
|-------|------|------|------|------|------|------|------|------|
|       | Mean | S.D. | Mean | S.D. | Mean | S.D. | Mean | S.D. |
| GSH in plasma | | | | | | | | |
| Mean | 0.99a | 0.001 | 0.23d | 0.027 | 0.92a | 0.003 | 0.74b | 0.024 |
| S.D. | 0.01 | 0.003 | 0.004 | 0.000 | 0.004 | 0.004 | 0.012 | 0.002 |
| % change | ------- | -76.8 | -7.07 | 49.5 | -25.3 | -54.5 | -44.4 | -64.6 |
| GSH in lung | | | | | | | | |
| Mean | 6.41a | 0.023 | 3.73b | 0.025 | 5.08a | 0.024 | | 1.231d |
| S.D. | 0.009 | 0.009 | 0.064 | 0.004 | 3.30bcd | 0.003 | | 1.30f |
| % change | ------- | -80.8 | -20.7 | -48.5 | -34.6 | -52.6 | -41.8 | -63.0 |

Values with different superscript letters were significantly different, P<0.05.

Exposure to cigarette smoke decreased the glutathione (GSH) values both in plasma and lung tissues by 76.8% and 80.8%, respectively (Table 7).

The impact of inducing the high dose of vitamin C on the GSH values was more effective than the low dose either before, during, or after smoke exposure.

As a result, it can be suggested that exposure to passive cigarette smoke cause oxidative stress and vitamin C may prevent the body cell from damaged.

The results of the present study are in agreement with those of (Passamai et al., 2010), the oxidative stress biomarkers were measured in individuals exposed to passive smoke before and after supplementation with vitamins C (500 mg) for six months and compared to a control group, they concluded that vitamin C supplementation was effective in decreasing markers of lipid and protein damage and improved both enzymatic and non-enzymatic antioxidant defenses.

Reduced glutathione (GSH) is one of the most abundant intracellular thiols, and aids in protection of cells from the lethal effects of toxic and carcinogenic compounds as well as a wide variety of drugs (Townsend et al., 2003)(Go& Jones, 2010). GSH can function as an antioxidant in the ingest free radicals, can maintain ascorbate in a reduced and functional form (Ortega et al., 2011). Therefore, GSH depletion may promote tumor development through a mechanism that involves cytotoxicity and other different ways (Sheweita & Tilmisany, 2003).

Glutathione is present in two forms, one is called reduced form (GSH) and the other form is the oxidized form (GSSG). Many toxic compounds may eventually lead to glutathione oxidation (GSSG) which changed into its reduced form by glutathione reductase (GR) activity (Petrulea et al., 2012).

These results agreed with those of (Ghoneim et al., 2015) who found higher levels of antioxidants in healthy plasma compared with patients with chronic obstructive pulmonary disease.

Histological results

Group 1 (Control): Light micrograph of control group mice section of lung Figure (1), showing a part of respiratory bronchiole (RB) with single columnar lining epithelium (SC). Alveoli (A) appear patent with thin wall linked by type I squamous epithelium (P1) and type II pneumocystis (P2). Thin inter-alveolar septum was noticed (†). Some small blood vessels (BV) reveal congestion with RBCs.

Group 2 (SMOK): Figure (2) shows congested thick walled blood vessels (BV), cellular infiltration I, and stratification collapsed respiratory bronchiole (RB). Alveoli are patent with thick wall and some shows secretions with their lumen (†).

Group 3 (HCBS): Photomicrograph of group(CBSH) mice lung showing mild peri-bronchiole, cellular infiltration, and patent alveoli with thin IAS (†) as shown in (Fig 3).

Group 4 (LCBS): Lung section shows patent alveoli with areas of thick IAS (†) extravasations of RBCs (R) congested this walled blood vessel (BV) (Fig 4).

Group 5 (HCDS): Photomicrograph of mice lung section shows patent alveoli (A), some showing thick inter-alveolar septum (†), dust and dark particles (D) were striking in this photo (Fig. 5).

Group 6 (LCDS): Section of mice lung reveals collapsed alveoli (A) lined by irregular wall, thick inter-alveolar Septum (†), some alveoli are completely obliterated with massive intercellular infiltration (I) (Fig 6).
Fig. 1. Light micrograph of control group mice section of lung

Fig. 2. Light micrograph of SMOK group mice section of lung

Fig. 3. Light micrograph of HCBS group mice section of lung

Fig. 4. Light micrograph of LCBS group mice section of lung

Fig. 5. Light micrograph of HCDS group mice section of lung

Fig. 6. Light micrograph of LCDS group mice section of lung

Fig. 7. Light micrograph of HCAS group mice section of lung

Fig. 8. Light micrograph of LCAS group mice section of lung
**Group 7 (HCAS):** Lung tissues show patent alveoli (A), while other are collapsed. Thick inter-alveolar septum (†) with cellular infiltration (I) esp. Neutrophil (Ns), RBCs extravasation between alveoli. Increased numbers of Phrenocytes II (P2) were noticed in the upper hyper-inflated alveolus (Fig 7).

**Group 8 (LCAS):** Lung section illustrates massive collapse of most of alveoli, thick IAS (†), congested blood vessel (BV), stratification of respiratory bronchiol (Rb) with exfoliation of some cells in its lumen (††) (Fig 8).

In this study, most bronchioles showed partial shedding of the mucous lining and the appearance of cellular residue inside. These cloud changes are due to the direct toxic effect of smoke on the bronchioles lining the mucosa. These results were consistent with those previously reported after cigarette smoking in terminal bronchioles, where incomplete areas of epithelial desquamation and accumulation of cellular debris within the cavity were detected (Dye & Adler, 1994).

The inflammatory cellular infiltration that was discovered in this study can be considered a lung defense mechanism against the toxic effect of air pollutants. Infiltration cells help the rapid and rapid removal of foreign particles such as tissue debris and red cells, paving the way for regeneration. The current data was more consistent with the view that cellular defensive reaction occurs primarily against pathogenic bacteria or irritating chemicals (Junqueira & Carneiro, 2003).

There is pulmonary congestion with the thickness and expansion of the pulmonary vessels, the thickening of the barrier between the vessels by a marked infiltration of a single nucleus cell including macrophages with blood leaking into the lumen of some of the pulmonary vesicles. Camouflage of most air sacs was detected, then the adjacent parts were stretched and compensated with the alveolar wall destroyed with the increased deposition of collagen fibers in the interstitial, around the bronchi and pulmonary vessels.

The pulmonary vascular congestion observed in rats exposed to tobacco smoke may be from un filtered cigarettes due to the toxic effects of smoke. Smoking may affect blood vessels by releasing vasodilators into the bloodstream. Stagnant blood in dilated capillaries will cause hypoxia in the lung tissue resulting in greater pulmonary congestion (Gilman et al., 1981).

Interstitial bleeding as well as within the alveoli in these mice can be explained by increased vascular permeability. Vascular permeability was a result of the release of polypeptide mediators from smoke-exposed cells (Shoji et al., 1995). In addition, cigarette smoke has impaired endothelial function in smokers due to increased oxidative stress and enhanced formation of free radicals derived from oxygen (Motoyama et al., 1997).

Toxicity is observed directly on the capillary wall leading to ischemia, followed by vasodilation and blood escaping from its dead wall to the barrier between the vesicles and the lumen of the alveoli. This was similar to the result that long-term exposure to nitrous oxides as an air pollutant showed significant lung congestion (Nakai et al., 1999). Moreover, lung congestion was usually associated with a decrease in gas exchange resulting in an expansion of the alveolar space in the air (Carpy et al., 2000).

Disruption and injury to the blood vessel wall may be explained by the relative hypoxia caused by exposure to carbon monoxide (CO) (Holley et al., 1999).

The increased thickness of the walls between the follicles observed in the experimental mice of this study can be explained by the presence of excess inflammatory cells, capillary congestion, increased interstitial connective tissue and the associated alveolar collapse. These results coincided with those who linked the thickness of the alveolar septum with a modification in the blood vessels leading to infiltration and inflammatory edema (Hora et al., 2003).

The current study showed the destruction of some alveolar walls and foci of collapsed vesicles with the subsequent expansion of adjacent alveolar spaces and the formation of large irregular areas (maternal changes). These changes were previously described by researchers who transmitted this result to lung tissue injury by oxidative mechanisms created by oxidants in cigarette smoke extracts as well as those released by specific inflammatory cells, especially alveolar macrophages and neutrophils (Czekaj et al., 2002).

Important role was played by macrophages in the cause of smoke emphysema. The elastic enzymes released by macrophages have damaging effects on the airway wall. Exposure to chronic smoke increases the production of metallo-proteinases (MP) by macrophage. These are proteolytic enzymes and their enhanced release may be responsible for the destruction of lung tissue (Barnes et al., 2003) (Stewart & Voelkel, 2008).

**CONCLUSION**

The results indicated the following:

1. Exposure to smoke led to doubling of the relative weight of the lung.
2. Exposure to smoke led to decrease the vitamin C levels in plasma.
3. Plasma and lung TBARS increased by exposure to smoke.
4. Plasma and lung GSH decreased by exposure to smoke.
5. Vitamin C high dose intake showed protective effect against the negative impacts of smoke.
6. Vitamin C high dose intake showed a relative improvement in the histology of the lung.

**SIGNIFICANCE STATEMENT**

This study indicated that vitamin C increases the ability to reduce the risk of passive smoking, especially high doses of vitamin C (1 g / day for human) had a protective effect on respiratory health, it helps to reduce the prevalence of chest diseases caused by air pollution. The recommended dose in the study is able to protect the lung from the harmful effects of smoking. Whereas, this dose of vitamin C (1 g per day) is unable to repair damaged lung cells. So, this study may help researchers to estimate another dose of vitamin C as cured dose to repair this damage.

**RECOMMENDATIONS**

1) The importance of having (3 servings/ day) of fresh vegetables at least, especially sweet peppers, leafy vegetables and tomatoes as rich sources of vitamin C. For example, eating three servings or three cups of chopped sweet pepper daily give approximately 500 mg of vitamin C.
2) The importance of intake (3 servings/ day) of fresh fruits at least, especially, guava, kiwi, strawberries and citrus fruits. For example, eating three servings of guava a day will give approximately 684 mg of vitamin C.
3) Avoiding exposure to all sources of air pollution especially passive smoking.

**ACKNOWLEDGMENT**

Praise to "Allah", the Most Gracious and the Most Merciful Who Guides Us to the Right Way. All authors would like to acknowledge the Department of Home Economics, Faculty of Agriculture, Histology Department, Faculty of medicine and Institute of Graduate Studies and Research - Alexandria University for providing laboratory facilities for the research study.

**REFERENCES**

ALA , American Lung Association. 2010. State of Lung Disease in Diverse Communities. www.lungusa.org.
Barnes, P.J., S.D.Shapiro and R.A.Pauwels. 2003. Chronic obstructive pulmonary disease: Molecular and cellular mechanisms. Eur. Respir.J.22(4):672-688.
Berthon, B.S. and L.G.Wood. 2015. Nutrition and Respiratory Health–Feature Review. Nutrients. 7: 1618-1643.
Candon, S.P., Battlehner, C., Lorenzi-Filho, G., Dohlnikoff, M., Pereira, P.M., Concejiao, G.M., Beppu, O.S., and Saldiva, P.H. 1995. Pulmonary emphysema induced by passive smoking: An experimental study in rats. Brazilian J. of Medical and Biological Research. 30: 1241-1247.
Carpy, S.A., W.Kobel and J. Doe. 2000. Health risk of low dose pesticides mixtures: A review of the 1985-1998 literature on combination toxicology and health risk assessment. J.Toxicol. Environ.Health, Part B. Crit. Rev. 3(1):1-25.
CDCP, Centers for Disease Control and Prevention. 2012. Fact Sheet - Secondhand Smoke Facts - Smoking & Tobacco Use. Cdc.gov.
CDCP, Centers for Disease Control & Prevention Fact Sheets, 2013. Tobaccofreefloridanewsroom.com
Childs, A.C., S.L.Phaneweuf, A.J.Dirks, T.Philips and C.Leeuwenburgh. 2002. Doxorubicin treatment in vivo causes cytochrome C release and cardiomyocyte apoptosis, as well as increased mitochondrial efficiency, superoxide dismutase activity, and Bcl-2: Bax ratio. Cancer Res. 62: 4592-4598.
Czekaj, P., A.Palasz, T.Lebda Wyborny, G.Nowaczyk Dura, W.Karczewska, E.Florek and M.Kaminski. 2002. Morphological changes in lungs, placenta, liver and kidneys of pregnant rats exposed to cigarette smoke. Int. Arch. Occup. Environ. Health. 75 Suppl: S27-S35.
Doll, R., R.Peto, J.Boreham and S. Sutherland. 1994. Mortality in relation to smoking: 50 years' observations on male British doctors. BMJ (Clinical research ed.)328 (7455): 1519.
Drury, R.A.B. and E.A.Wallington. 1980. Carlton's histological technique. 5th ed. Oxford, New york, Toronto: Oxford University. 140-142.
Dye, J.A. and K.B.Adler. 1994. Effects of cigarette smoke on epithelial cells of the respiratory tract. Thorax. 49(8):825-834.
Ghoneim, A.H., M.A.Al-Azzawi, S.A.Elmasry, M.Y.Nasr and M.M.N.AboZaid. 2015. Association of vitamin D status in the pathogenesis of COPD. Egyptian J. of Chest Diseases and Tuberculosis.
Gilman, M.J., J.T.Sylvester, T.P.Kennedy, H.A.Menkes and R.J.Traystman. 1981. Vascular effects of cigarette smoke in isolated pig lungs. Am. Rev. Respir. Dis. 124(5):549-553.
Go, Y.M., D.P.Jones. 2010. Redox control systems in the nucleus: mechanisms and functions. Antioxid Redox Signal. 13(22):489–509.
Hemila, H. 2004. Vitamin C Supplementation and Respiratory Infections. Systematic Review. Mil Med. 169(11):920-5.
Holley, J.E., J.W.Butter and J.M.Mahoney. 1999. Carbon monoxide poisoning in racing car drivers. J. Sports Med. Phys. Fitness.39(1):20-23.
Hora, K., A.Fonscra, S.S.Valencia and R.Stanos. 2003. Lung Morphology in Rats Treated With Inraperitoneal Nicotine. Acta microscopica. 12:331-332.
Huy, L.A., H.Hua and C.P.Huyc. 2008. Free Radicals, Antioxidants in Disease and Health. International J. of Biomedical Sci. 4(2): 89-96.

Junqueira, L.C. and J.Carneiro. 2003. The respiratory system. In: Anonymous Basic histology: Text and atlas.10th ed.: Lange Medical Books McGraw-Hill, USA. 349-367.

Kelly, F.J. 2005. Vitamins and respiratory disease: Antioxidant micronutrients in pulmonary health and disease. Proc. Nutr. Soc. 64: 510–526.

Motoyama, T., H.Kawano, K.Kugiyama, O.Hirashima, M. Ohgushi, M.Yoshimura, H.Ogawa and H. Yasue. 1997. Endothelium-dependent vasodilation in the brachial artery is impaired in smokers: Effect of vitamin C. Am. J. Physiol. Heart Circ. Physiol. 273(4):H1644-H1650.

Nakai, S., J.S.T. Crest, H.Nitta and K.Maeda. 1999. Respiratory health associated with exposure to automobile exhaust. III. Results of a cross-sectional study in 1987 and repeated pulmonary function tests from 1987 to 1990. Arch. Environ. Health. 54(1):26-33.

NCI, (National Cancer Institute). 2007. Antioxidants and Cancer Prevention, Fact Sheet. Archived from the original on 4 March 2007.

Ortega, A.L., S.Mena and J.M.Estrela. 2011. Glutathione in cancer cell death. Cancers (Basel). 3(26):1285–310.

Petrulea, M., A.Muresan, I.Duncea. 2012. Oxidative stress and antioxidant status in hypo- and hyperthyroidism. In: Intech. 197–236.

Possamai, F.P., S.A.Júnior, E.B.Parisotto, D.W.Filho. 2010. Antioxidant Intervention Compensates Oxidative Stress in Blood of Subjects Exposed to Emissions from A Coal Electric-Power Plant in South Brazil, Federal University of Santa Catarina, Source: PubMed, https://www.researchgate.net/publication/51519721.

SAS, Statistical Analysis System, 1986. User's Guide: Statistics. Version 5 Edition. Inst.Inc.Cary.NC.U.S.

Seget, M., D.Karloczak, M.Wilk, A.Błaszczyk, L.Szyliberg, E.Florek and A.Marszałek. 2012. The awareness of carcinogenic effect of tobacco smoke—a questionnaire survey of students and employees of Collegium Medicum of Nicolaus Copernicus University. Prz. Lek. (in Polish). 69 (10): 904–7.

Sheweita, S., K.Tilimsany. 2003. Cancer and phase II drug-metabolizing enzymes. Curr Drug Metab. 4(13): 45–58.

Shoji, S., R.F.Ertl, S.Koyama, R.Robbins, G.Leikauf, S.Von Essen and S.I.Rennard. 1995. Cigarette smoke stimulates release of neutrophil chemotactic activity from cultured bovine bronchial epithelial cells. Clin. Sci. 88(3):337-344.

Steel, R.G.D. and J.H. Torrie. 1981. Principle and Procedures of statistics. A biochemical Approaches, 2nd ed.McGraus-HillsBook Company, New York, USA. 281-300.

Stewart, L. and N.F.Voelkel, 2008. Molecular pathogenesis of emphysema. J. Clin. Invest. 118(2): 394-402.

Townsend, D.M., K.D.Tew, H.Tapiero, 2003. The importance of glutathione in human disease. Biomed Pharmacother. 57(10):145–55.

Woodside, J.V., D.Call, C.Garcia-Parado, P.C.Gibson, P.G.Gibson. 2005. Airway and Circulating Levels of Carotenoids in Asthma and Healthy Controls. J. Am. Coll. Nutr. 24: 448–455.
الملخص العربي

الجرعة الفعالة من فيتامين ج لمواجهة التدخين السلبي

سهير فؤاد نور، تيسير ممتاز العصار، نهى محمود زهران

نظراً لدور الهم الذي يلعبه فيتامين ج في جسم الإنسان والذي يحمي الجسم من التأثيرات الضارة للشوارد الحرة وخاصة التي تكون نتيجة التدخين السلبي فقد استهدفت الدراسة معرفة الجرعة الفعالة من فيتامين ج والتي قد تكون لها دور في حماية الرئة من التأثيرات الضارة للتدخين السلبي، وذلك من خلال اختبار تأثير جرعتين محددتين من فيتامين ج على فئران التجارب (0.075 - 0.015 ملمجم) قبل وأثناء وبعد التعرض للتدخين السلبي والتي تعادل 200 ملمجم و1000 ملمجم من فيتامين ج على التوالي يومياً للإنسان.

وفد كان للجرعة الكبيرة من فيتامين ج في جسم الإنسان والذي يحمي الجسم من التأثيرات الضارة للشوارد الحرة وخاصة التي تكون نتيجة التدخين السلبي، حيث أن الجرعة الفعالة من فيتامين ج يمكن أن تكون من 0.075 - 0.015 ملمجم من فيتامين ج كمية تصل إلى 200 ملمجم و1000 ملمجم، وذلك من خلال زيادة المتناول من الأغذية الغنية فيتامين ج مثل الخضروات والفاكهة بالحصص المقررة في توصيات الدراسة.

ولهذا كان من الضروري حماية الرئة من التأثيرات الضارة للتدخين السلبي بزيادة الجرعة اليومية للإنسان لتصبح إلى 1000 ملمجم، وذلك من خلال زيادة المتناول من الأغذية الغنية فيتامين ج مثل الخضروات والفاكهة بالحصص المقررة في توصيات الدراسة.