Original Article

Propolis Alleviates Chromium Intoxication Of The Lung In A Rat Model: Histological, Immunohistochemical, And Biochemical Study

Dr. Ghada Nady Ouais[1,2] ,and Dr. Doaa Mohamed Hassan [3]

[1]Lecturer of Anatomy and Embryology, Faculty of medicine, Cairo University. ghada.nady@kasralainy.edu.eg. 01225320845.
[2]Lecturer of Anatomy and Embryology, Faculty of medicine, New Giza University. ghada.nady@ngu.edu.eg. 01225320845.
[3]Lecturer of Anatomy and Embryology, Faculty of medicine, Beni-Suef University. Doaa_eldary333@yahoo.com. 01003806640.
*Corresponding author: Ghada Nady Ouais
Address: Kasralainy faculty of medicine, Cairo university
E.Mail: ghada.nady@kasralainy.edu.eg
Orcid number: 0000-0002-6224-601X
DOI: 10.47750/pnr.2021.12.02.34

Abstract

Introduction: Chromium [Cr (VI)] was considered as one of the most famous cytotoxic environmental pollutants. It was believed to be a health harmful agent in cumulative way. Natural Propolis was thought to possess anti-inflammatory, anti-cytotoxic, and antioxidant abilities.
Objective: The purpose of this study was to assess Propolis' ability to protect rats' lungs from chromium-induced lung damage.
Material and Methods: Four equal groups of twenty-four adult male albino rats were established: Group I: served as control received normal saline. Group II (Propolis group): treated with 50 mg/kg/day of Propolis orally through gastric tube for four weeks. Group III [Sodium Dichromate Cr (VI) group]: received sodium dichromate intraperitoneal (i.p.) over eight days, at dosages of 12 mg/kg every day. Group IV were pretreated with Propolis for four weeks before administration of Sodium Dichromate Cr (VI) for eight sequential days at previous mentioned doses. At the end of experiment the animals were sacrificed after anesthesia and their lungs were removed and processed for biochemical, histological, and immunohistochemical investigations.
Results: Sodium dichromate Cr (VI) produced distortion of bronchial mucosa, inflammatory cellular infiltration, and congestion of blood vessels. Biochemical markers showed significant induction of malondialdehyde (MDA) levels indicating lipid peroxidation, significant decrease of glutathione (GSH) and superoxide dismutase (SOD) levels indicating oxidative effect in group III in contrast to the control group. The total area percentage of collagen fibers and the positive caspase-3 immunostained cells were significantly considerably higher in group III when compared to the control group. The administration of Propolis showed obvious improvement of these pulmonary alterations.
Conclusion: Exposure to Cr (VI) induced lung intoxication in the form of structural changes of the lung tissue. Collectively, the present study suggested that Propolis allows pulmonary protection against the inflammation and apoptosis caused by Chromium.

Key words: Propolis, Chromium, Lung toxicity.

Introduction

A heavy metal that is naturally found in the environment is Chromium. The hexavalent chromium compounds [sodium dichromate Cr (VI)] despite being extremely harmful, it typically used in various industries **(1&2).** Cr (VI) in small amounts enters the body through the gastrointestinal system, lungs, and skin. Long-term exposure to this metal is linked to serious harm to the kidneys, liver, intestinal tract, respiratory system, and the heart **(3&4).** Both human cytotoxicity and genotoxicity of this component have been described by **Li et al [5].** Mice that consume large amounts of Cr (VI) in water over time develop intestinal adenomas and carcinomas. After only one week of exposure, it damages the intestinal villi, and inducing crypt hyperplasia in mice **(3& 6). Kumar and Gangwar, [7]** reported that Cr (VI) caused hepatocellular and renal injuries**.** Liver and kidney's oxidative stress and lipid peroxidation (LP) caused by exposure to Cr (VI) in rats has been reported **(8).** In liver studies, **Xiao et al., [8]** shown that Cr (VI) also caused mitochondrial damage **(8).** The respiratory system was primarily impacted by the Chromium inhaled. In both people and animals, exposure to it increases the risk of pulmonary damage **(4, 9 &10).** Cr (VI) has the potential to worsen the antioxidant defense system, according to **Asatiani et al., [11]**. Although the specific role of Cr (VI) compounds in pulmonary toxicity is unknown, chromates are quickly absorbed by cells and converted into reactive Cr species, which may also result in the production of reactive oxygen species (ROS) **(11&12).** Rapid intracellular conversion of Cr (VI) to Cr (III) results in apoptotic alterations via several routes **(11& 12).** Interestingly there is a commercial attention of Propolis usage due to its therapeutic potentiality in a variety of diseases such as diabetes, skin wounds, gastrointestinal and cardiovascular disease (**13).** Propolis is regarded as an antioxidant, antibacterial, and

anti-inflammatory component. It modulates the immune system and has topical anesthetic influence and the ability to improve wound healing process. It decreases the prevalence of dental caries **(14).** Its anti-inflammatory ability is due to its modulation of cytokines and inflammatory mediators and inhibiting the production of prostaglandins **(14).** Propolis reduces vascular permeability via restraining the mast cells releasing power of histamine **(15).** Propolis is believed to be a source rich in natural antioxidants and used as a protective component against many free radical-associated degenerative diseases. Its antioxidant ability is achieved by reducing malondialdehyde (MDA) levels and inducing glutathione peroxidase (GPx) and superoxide dismutase (SOD) levels. Also, caffeic acid phenethylester (CAPE), a propolis component reduces cellular apoptosis by restricting the productions of caspase-3, nitric oxide synthase and cytochrome C **(16).**

Materials and methods

Drugs and chemicals

Sodium dichromate: obtained from Sigma-Aldrich Chemical Co. (USA).

Propolis purchased from Shana Honey Company in Egypt in a package of five grams of powder. Fifty milliliters of distilled water were used to dissolve the powder, and each milliliter contained 50 milligrams of Propolis.

Animals

Twenty-four adult male albino rats weighing between 150 and 200 grams were chosen for this study. They were obtained from the Animal House at Cairo University's Faculty of Medicine and were handled according to the standard instructions of Institutional Animal Care and Use Committee. In the animal house, the animals were acclimated for two weeks before commencing the experiment. They were provided with unlimited access to standard food and water while being housed in cages with standard laboratory and environmental conditions.

Experimental design

The animals were haphazardly divided into four equal groups, 6 rats/ cage and treated as follows:

Group I: The control group was given an oral dose of 0.9% saline solution.

Group II (Propolis group): for four weeks, Propolis was administered orally via gastric tube at a dose of 50 mg/kg/day **(17).**

Group III [Sodium Dichromate Cr (VI)]: Received sodium dichromate Cr (VI) intraperitoneal (i.p.) at dose of 12 mg/kg/day, for eight days **(18).**

Group IV: (sodium dichromate + Propolis) pretreated with Propolis for four weeks before administration of Cr (VI) for eight consecutive days at previous mentioned doses.

Lastly, a local anesthetic cream was applied to the animals' abdominal skin 30 minutes before the scarification; the animal has i.p. injection of a lethal dose of phenobarbitone sodium (80µg/kg). The lungs were dissected, and the right lungs were prepared for histological examination while the left lungs allowed for biochemical study.

Biochemical analysis:

MDA (lipid peroxidation marker), GSH and SOD (antioxidant enzymes) were assessed consuming commercially available ELISA kits accepting the manufacturer's instructions **(19).**

Histopathological study

After immediately fixed the lung specimens in 10% buffered formalin for approximately for three hours, the specimens were washed, dehydrated and then overnight embedded in liquid paraffin wax to make paraffin blocks, afterward by the microtome steel knife, sections of 5µm thickness were obtained then fixed on glass slides **(20).** The sections were consumed for histological study using Hematoxylin and eosin (H&E), Masson's trichrome staining and immunohistochemical study.

Immunohistochemical Study

The staining of the immunohistochemistry was carried out using the streptavidin-biotin-peroxidase technique **(21).** To identify cellular apoptosis, a 1/1000 dilution of an anti-caspase-3 rat monoclonal primary antibody (Dako Cytomation, Heverlee, Belgium) was used as a marker. Subsequently, the sections underwent deparaffinization, hydrated and washed in phosphate buffer saline

(PBS). Peroxidase blocking solution was used to block peroxidase then washed in tri-buffer saline (TBS). After that, the sections were treated with Caspase-3 (1:200) diluted primary antibodies. Sections are then washed in buffer and incubated with biotinylated goat anti-rabbit secondary antibodies for a further half an hour. Adding diaminobenzidine tetra hydrochloride (DAB) in distilled water for about five to ten minutes was done. Finally, the slides were then cleaned with xylene, dehydrated with increasing alcohol concentrations, and counterstained with Mayer's hematoxylin. As a positive control for active Caspase-3, tonsils were used.

Morphometric study

Quantitative data were exhibited using the Leica Qwin 500 Image Analyzer software system (National Institute of Mental Health, Bethesda, Maryland, USA). The sections were assessed using a standard measuring frame of 11694 µm2, for the known area. The final step of analysis was the abstraction of quantitative information from images. For every animal group, the parameters were inspected using an X40 objective lens in ten non-overlapping fields: (1) the thickness of the inter-alveolar septa (2) the percentage of the total area of collagen fibers within the Masson's trichrome-stained sections. (3) The immunoreactive positive cells in caspase-3-stained sections.

Statistical analysis

Microsoft Excel worksheet from 2010 was used to state the biochemical levels as well as the numerical data from the morphometr ic investigation. The Statistical Program for Social Sciences (SPSS) version 21.0 (IBM Corporation, Somers, NY, USA) was used to carry out the statistical analysis. The means ± standard deviation (SD) was displayed for the results. The statistical assessment was carried out using one-way evaluation of variance (ANOVA). Through the investigation, significance was determined when the p-value was less than 0.05.

Results

Biochemical results

Illustrated in **(Tab.1)** and **(Fig.1: a, b, c),** In comparison to the controls, group II (Propolis group) had insignificant changes in MDA, GSH, and SOD levels ($P>0.05$). Group III (Sodium dichromate treated group) exhibited a significant decrease in the levels of GSH and SOD and a significant increase in the level of MDA as compared to the control group, representing lipid peroxidation and oxidative pulmonary injury. Additionally, as compared to group III, group IV (sodium dichromate + Propolis) showed a substantial decrease in MDA levels and a significant increase in antioxidant enzyme levels ($P<0.05$), indicating the antioxidant capabilities of Propolis.

Histological results

The H&E-stained sections of both groups I, II (control, Propolis groups) respectively were the same and presented normal histological picture of the lung tissue. The sections revealed the alveoli of variable sizes, alveolar sacs where many alveoli open into, bronchioles and blood vessels. The alveoli were separated by thin inter-alveolar septa, lined by two types of cells; type I pneumocytes which are flattened squamous cells, and type II pneumocytes which are cuboidal cells. **(Fig.2: A, B, C and D**).

Group III (Sodium dichromate treated group) revealed histological changes comparable to control group. There were visible congested blood vessels and collapsed alveoli. Cellular debris was seen in the lumen of the disturbed bronchiolar epithelium. There was evidence of inter-alveolar septal thickening. Perivascular fibrosis, extravagated blood cells, and inflammatory mononuclear infiltration were seen. **(Fig.3: E and F).**

Alveolar tissue in Group IV (Propolis and sodium dichromate-treated group) appeared fairly normal. Although regions of some mononuclear infiltration remained, there was a relative decrease in the thickness of the inter-alveolar septa. Alveoli with wide spaces, lined by type I and type II pneumocytes were observed **(Fig.4: G and H).**

Masson's trichrome staining sections in both groups I, II (control, Propolis groups) presented normal appearance of collagen fibers arrangement surrounding the walls of bronchiole and blood vessels **(Fig.5: A and B).** Although group III, (sodium dichromate treated group) presented dense collagen fibers deposition surrounding the bronchioles and blood vessels **(Fig.5: C and D).** Furthermore, group IV (Propolis and sodium dichromate-treated group) showed a reduction of collagen fibers aggregation encircling the bronchiole **(Fig.6: E and F).**

Regarding lung sections immunostained with caspase-3, both groups I and II (control and Propolis groups) revealed negative immune reactions in the lung tissue **(Fig. 7: A and B).** While group III, which received treatment with sodium dichromate, showed

obvious evidence of apoptotic alterations with significant positive cytoplasmic caspase-3 immune expression **(Fig. 7:C).** Furthermore, group IV (which received Propolis plus sodium dichromate treatment) showed minimal caspase-3 expression in lung tissue **(Fig. 7: D).**

Morphometric and Statistical results

Parameters results in **(Tab.2)** and **(Fig.8: a),** revealed that group III (sodium dichromate treated group) displayed significant increase in the thickness of the inter-alveolar septa (P<0.001) as compared with the control and Propolis groups. However, group IV (Propolis and Sodium dichromate-treated group) revealed significant changes as compared with (group III) sodium dichromate treated rats.

Parameters results in **(Tab.2)** and **(Fig.8: b),** exhibited that group III (sodium dichromate treated group) demonstrated significant increase in the mean area % of collagen fibers aggregation comparable to both group I and II (P<0.001). Instead, group IV (Propolis and sodium dichromate-treated group) exhibited a significant regression in the mean area % of collagen fibers aggregation comparable to group III.

The area % of Caspase-3 immunoreactive expression in stained sections **(Tab.2)** and **(Fig.8: c)**, there was a significant induction in the expression area was observed in groups III (sodium dichromate treated group) as compared to groups I and II. However, in the group IV (Propolis and sodium dichromate-treated group) there was a significant decline in expression area comparable to group III.

Table 1: the biochemical enzymatic activities in all groups

Variable	Group I	Group II	Group III	Group IV
MDA (nmol/ml)	9.81±0.2	10.81±0.2	16.56±1.3*	12.57±0.4#
GSH-px (mU/mL)	46.8±6.4	48.8±6.4	13.6±2.6*	41.0±7.5#
SOD (U/ml)	132.6±1.7	133.6±1.7	78.64±6.1*	127.0±4.7#

Data were expressed as Mean ± SD, p value <0.05 was significant, (*) Denotes significant difference versus Group I and II, (#) Denotes significant difference versus Group III.

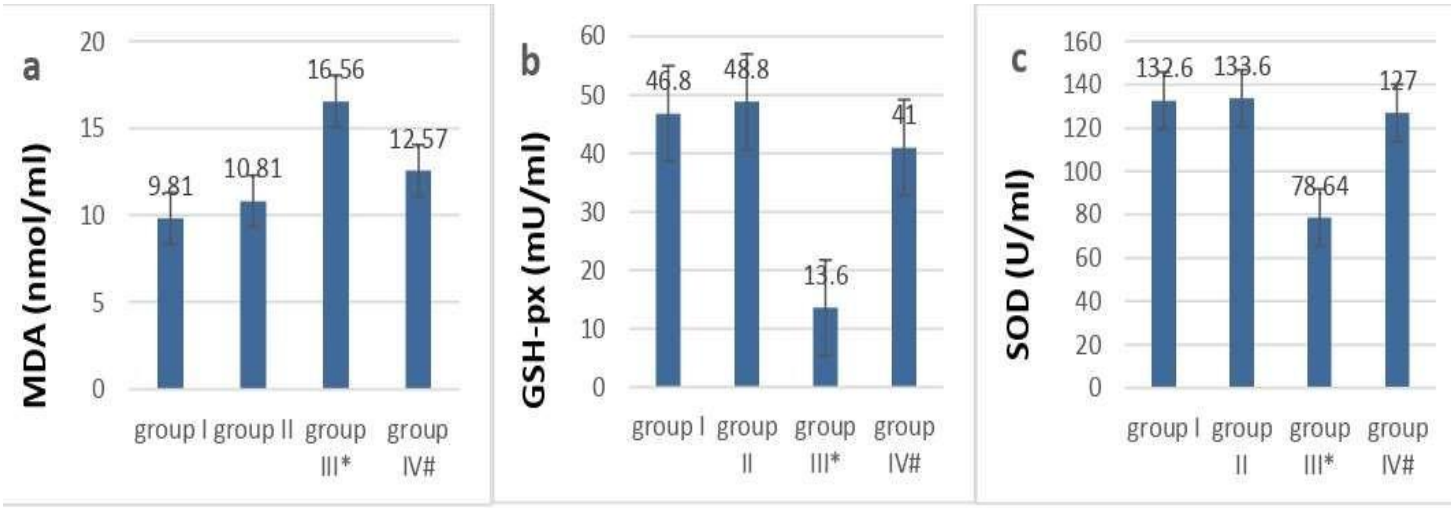

Fig. (1): Enzymatic activity of a: MDA (nmol/ml), b: GSH-px (Mu/ML) AND c: SOD (U/ml) in lung tissue of all studied groups. (*) Denotes significant difference versus Group I and II, (#) Denotes significant difference versus Group III.

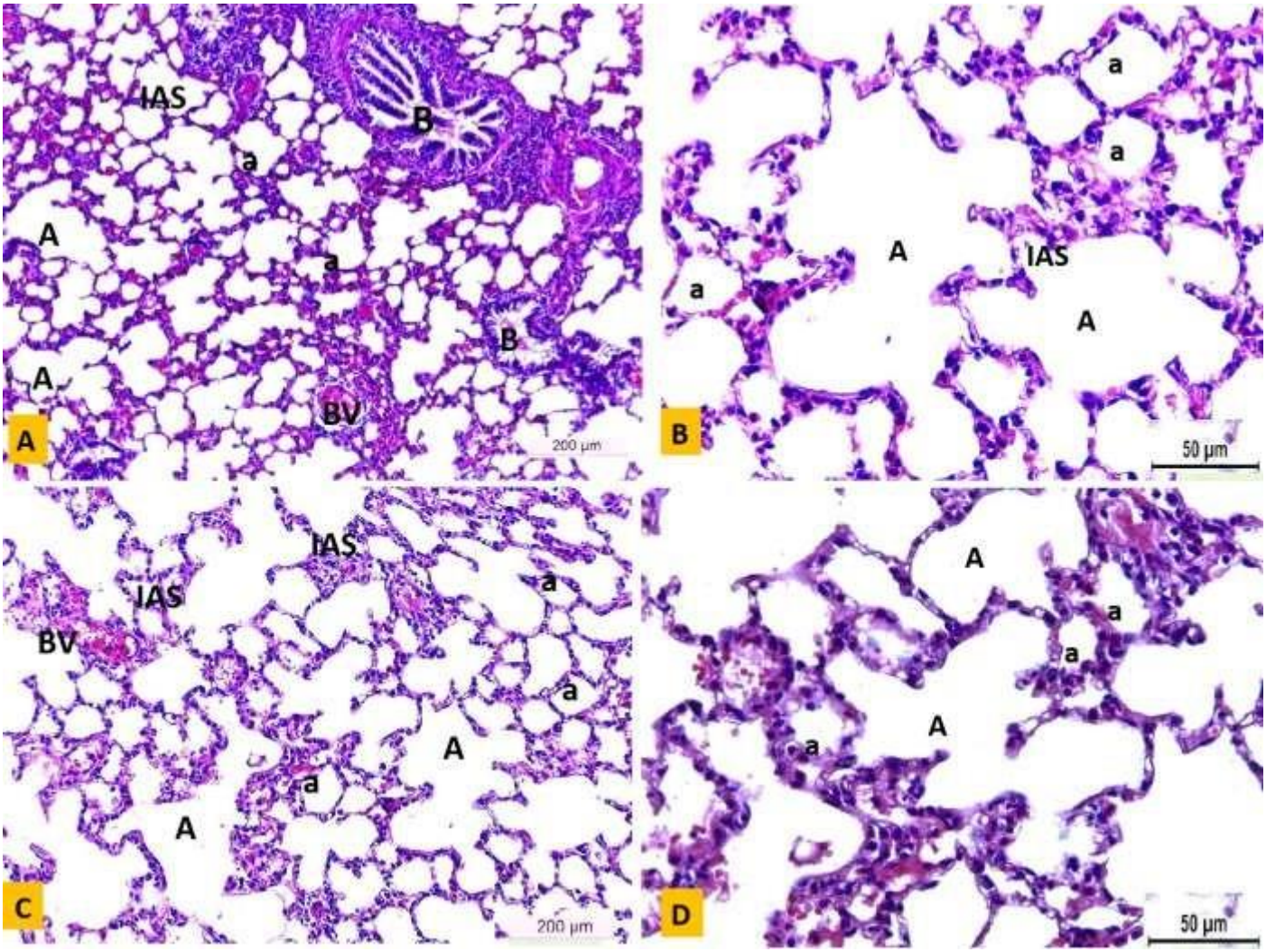

Fig. (2): H&E-stained sections of lung tissue. **Both A and B** Photomicrographs of lung sections from control group revealing alveoli (a), alveolar sacs (A), inter alveolar septa (IAS) blood vessel (BV) and a bronchiole (B) lined by columnar cells. **Both C and D** Photomicrographs of lung sections from Propolis group II that almost match the findings from the control group.

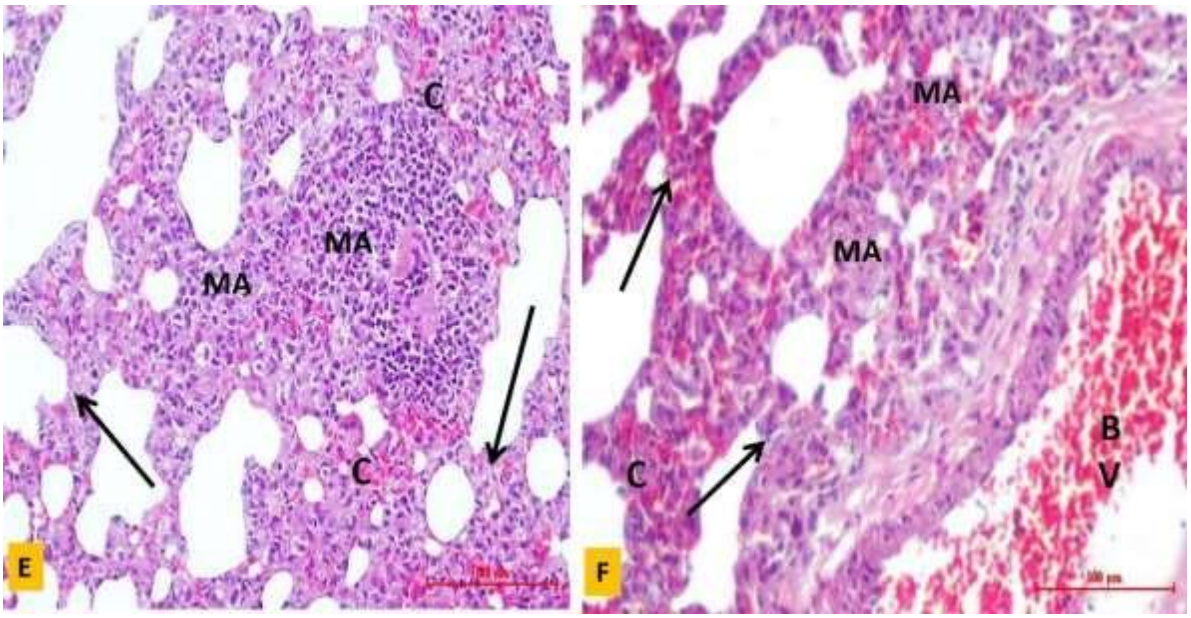

Fig. (3): H&E-stained sections of lung tissue. **Both E and F** Photomicrographs of lung sections from sodium dichromate group III illustrating the disruption of the wall and compression of the alveoli and alveolar sacs by a mononuclear aggregate (MA). Notice the distinct thickening of the IAS (arrows), extravasated RBCs (C) in some alveoli, and congested blood vessels (BV).

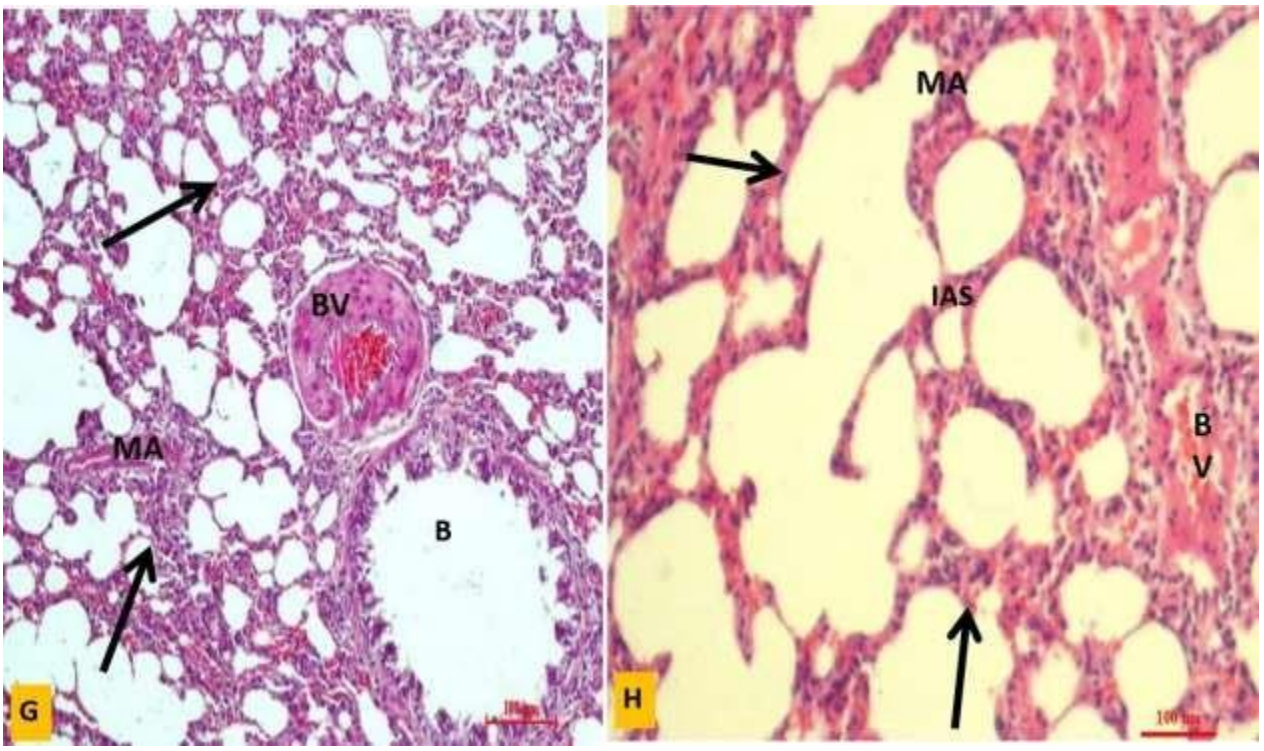

Fig. (4): H&E-stained sections of lung tissue. **Both G and H** Photomicrographs of lung sections from group IV (Propolis plus Sodium dichromate) displaying a bronchus with few shed epithelial cells (B) in the lumen and congested vessels (BV). Some IAS (arrows) exhibited thickening, and some mononuclear infiltrating cells were observed.

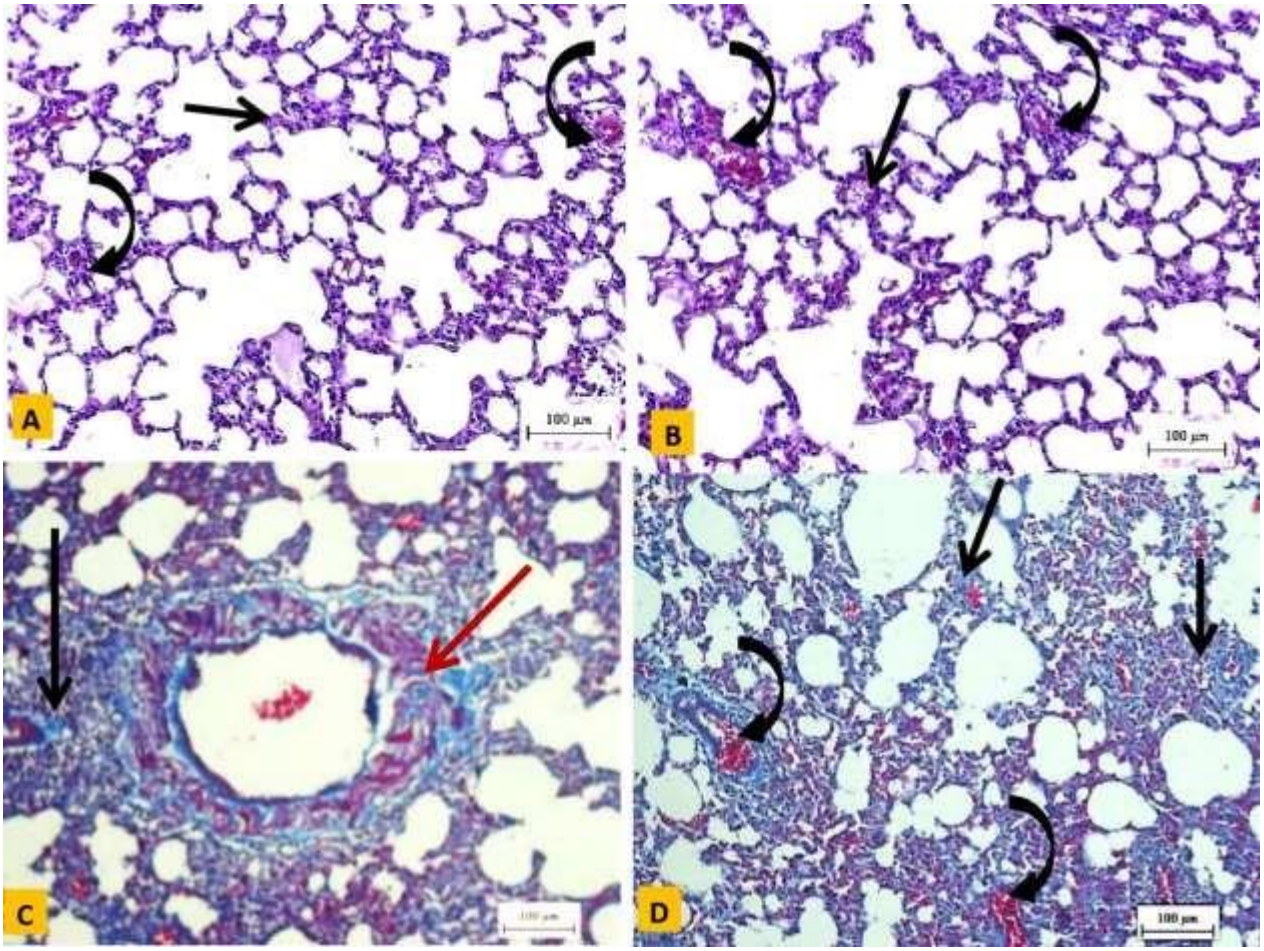

Fig. (5): Masson's trichrome stained lung sections. **Both A and B** lung sections from control group I and Propolis group II respectively showing few fine collagen fibers in the IAS (arrows) and in the adventitia of blood vessels (curved arrows). **Both C and D** lung sections from sodium dichromate group III illustrating deposition of numerous dense collagen fibers in thickened IAS (black arrows), in the adventitia of bronchioles (red arrow), in addition to that of congested blood vessels (curved arrows).

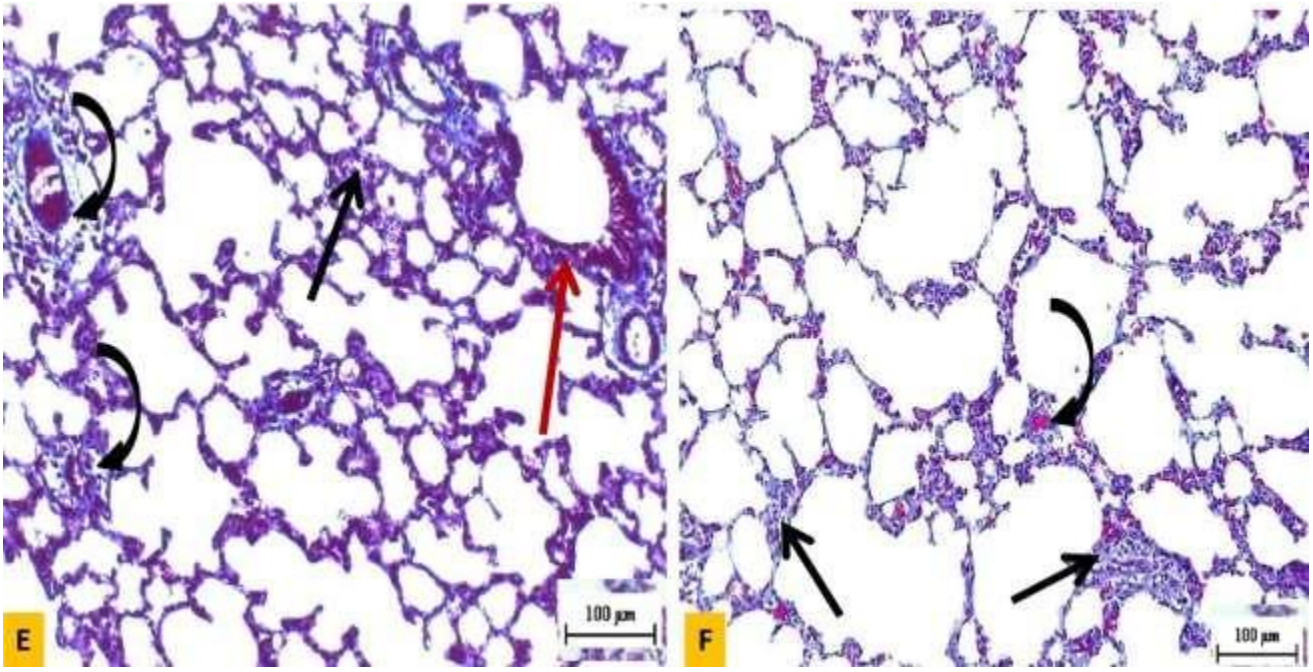

Fig. (6): Masson's trichrome stained lung sections. **Both E and F** lung sections from group IV (Propolis plus Sodium dichromate) showing some tiny collagen fibers in the IAS (black arrows) in the adventitia of blood vessels (curved arrows) in a bronchiole adventitia (red arrow) and in that of congested blood vessels (curved arrows).

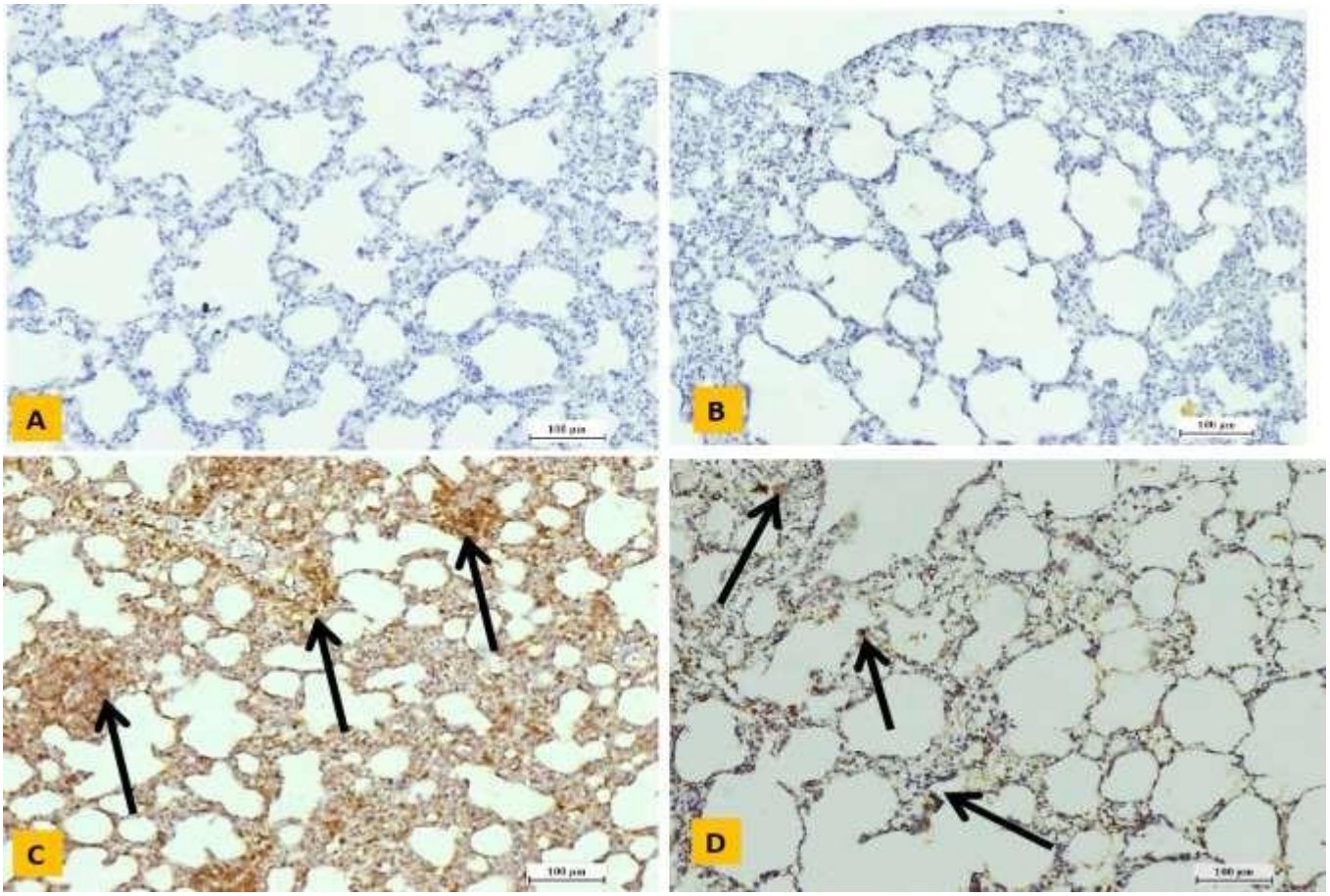

Fig. (7): Caspase-3 immunostained sections of lung tissue. **A and B:** the control group I and the Propolis group II respectively showing almost the same finding of normal immuno-expression of Caspase-3. **C**: sodium dichromate group III showing marked increase in Caspase-3 immunostaining particular in thickened interstitial tissue and numerous pneumocytes (arrows). **D**: group IV (Propolis plus Sodium dichromate) showing mild Caspase-3 immunostaining in pneumocytes in (arrows).

Table (2) the histomorphometric parameters in all groups:

Variable	Group I	Group II	Group III	Group IV
Thickness of interalveolar septum (µm)	90.7±1.0	92.5±2.0	363.4±4.6*	115.7±5.4 *#
Masson's trichrome Mean area %	3.7 + 0.232	2.5 + 0.145	12.2 + 0.5 11*	5.5 + 0.523*#
Caspase 3 Mean area %	1.5 + 0.412	2.3 + 0.2721	16.8 + 0.7 22*	9.5 + 0.514*#

Data were expressed as Mean ± SD, p value <0.05 was significant, (*) Denotes significant difference versus Group I and II, (#) Denotes significant difference versus Group III.

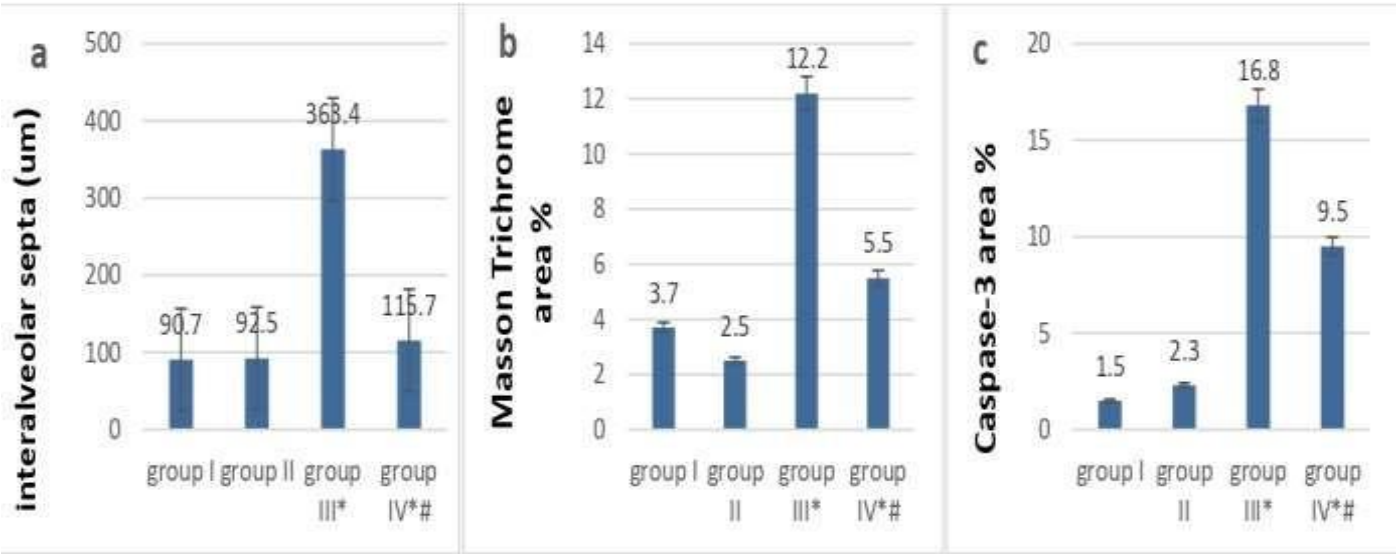

Fig. (8): a: Thickness of interalveolar septum (µm), b: Masson's trichrome Mean area %, and c: Caspase 3 Mean area % in lung tissue of all studied groups. (*) Denotes significant difference versus Group I and II, (#) Denotes significant difference versus Group III.

Discussion:

Currently materials containing Chromium are consumed in numerous industrial and chemical processes. This occupational contact to hexavalent chromium caused health hazards as one Chromium compound can manufacture sodium dichromate which has harmful consequences and produces ROS **(22).** Owing to the inequity between oxidation and antioxidant protections, sodium dichromate is known to be one of the pulmonary cytotoxic agents, causing alveolar matrix structure modification via the oxidative effect **(7).** The link between oxidative stress and the tissue toxicity initiated by contact to hexavalent Chromium indicates that using the antioxidant agents might diminish the toxicity of chromate-induced **(23).**

Presently the Propolis is widely consumed, due to its effects on many inflammatory responses, immunity and oxidative stress **(23).** The aim of this study was to discover a natural protective supplement to protect the lung tissue against damage and toxicity caused by sodium dichromate.

In the current study, the tissue oxidation and the apoptotic effects were detected after administration of sodium dichromate. These consequences were assessed by many methods such as histopathological, immunohistochemical and biochemical analysis.

In the current research, the group of animals treated with sodium dichromate developed significantly reduction in the antioxidant markers; GSH and SOD and significantly induction of the lipid peroxidation marker; MDA comparing to the control and Propolis only treated groups that obviously demonstrating pulmonary injury by lipid peroxidation, tissue oxidation. It was evidenced that a reduction of the activity of the measured antioxidant enzymes results in agreement with **Navya et al [23]**.

According to our conclusions, **Boşgelmez and Güvendík [24]** established that in drinking water the presence of Cr (VI) could decrease the antioxidant enzymes synthesis and activation **(24).** Harmonized with our results, **Caglieri et al [2] and Ambreen et al [25]** stated that the workers exposed to Cr (VI) had numerous cutaneous, pulmonary diseases plus changes in oxidative stress parameters **(2, 25).**

Contrary to our results, **Soudani et al [19]** stated that, the lung tissue did not show induction of oxidative stress as there were minimal tissue alterations. The later claimed that to the Cr (VI) concentration and the exposure route **(19).**

Iztleuov et al [26] supported our results as the consuming of the Boron compound to antagonize the toxicity and physio-biochemical alternations of Chromium VI that related to bronchopulmonary **(26).** In another investigation**, Iztleuov [26]** and his team declared the disturbance balance of the oxidant and antioxidant and increase ROS production due to the reduction of Chromium VI to Chromium III intracellular using Fenton reaction **(27, 28).**

In the current research, group III (sodium dichromate treated group) the lung tissue developed structural disrupt collapsed alveoli beside compensatory dilation of other alveoli. Also, the results showed congested blood vessels, injured bronchiolar epithelium with present of cellular debris in the lumen, the inter-alveolar septa are thickened, and inflammatory cellular infiltration were noticed. These outcomes were in harmony with the findings of **Soudani et al [19] and Marat et al [28]** who focused on the pathological outcome of Chromium on the lung in rats **(19, 23, 28).**

These pulmonary alterations could associate to the oxidative status, formation of ROS and excess generation of free radicals which are cytotoxic elements that damage cellular proteins, DNA and membrane lipids preceding to apoptosis and cell death, as described before **(29, 30).**

These elaborations of free radicals and ROS are owed to the oxidative condition produced by Chromium cause induction of proinflammatory cytokines that were directed to the harmful inflammatory changes discovered in the lung tissue in this research. These former findings were in accordance with research by **(30, 31).**

In the present study, in group III the significantly thickened interalveolar septa compared with the control and Propolis groups might the cause of excess infiltration of inflammatory cells such as macrophages and neutrophils, proved previously by **(18, 31).** The former authors stated that the Chromium encourage releasing of inflammatory cytokines, and their accumulation in the interalveolar septa.

In the current work, exposure to sodium dichromate caused significant increase deposition of collagen fibers surrounding the bronchioles, blood vessels and within the alveolar wall clearly detected in Masson's trichrome stained sections comparable with control and Propolis groups. These outcomes were in harmony with **Schneider et al [32]** who proved that the inflammatory response caused by Chromium consequences to activation of fibroblast and collagen deposition **(32).**

In the present work, apoptotic lung cells were noticed in group III (sodium dichromate). This finding was harmonized with the results of immunohistochemical assay as there was a significant increase in area percentage of the cellular expression of Caspase-3 which was in comparable to both control and Propolis groups. These findings were settled in previous studies by **(33, 34).** Exposure to Chromium increases Caspase 3 cellular expression, directing change in the permeability of mitochondrial membrane, following discharge of cytochrome c into cytosol and Caspase stimulation which on top directs to cellular apoptosis **(35).** Besides that, the oxidative stress headed to the cellular homeostasis disturbance with incidence of cellular degeneration and apoptosis. Consequently, the search for drugs based on substances with antioxidant impact is a basis for the protection and treatment of Chromium pulmonary intoxication.

In this study, group IV treated with Propolis showed obvious improvement in the biochemical, histological and immune-histochemical results. This means that the Propolis has a possible protective role against Chromium pulmonary intoxication. These discoveries were comparable to earlier research that expected that Propolis has a protective role against lung toxicity **(36).**

Propolis is one of the most hopeful natural supplements, with both curative and protective properties with no side effects. In agreement with the current work, **Korish and Arafa** [37] and **Araujo et al** [14] established that Propolis has anti-inflammatory properties against both acute and chronic inflammatory conditions when used in vitro and in vivo. The last authors **(37)** provided evidence for Propolis anti-inflammatory properties by demonstrating that it prevents neutrophil penetration and deactivates pro-inflammatory cytokines.

Propolis performances as anti-inflammatory and immunomodulatory agent by decreasing neutrophil infiltration, inhibiting proinflammatory cytokines, all of which imitate the immune defense **(15).** Moreover, Propolis has a potent antioxidant role by declining the cellular lipid peroxidation that was shown in this study by reducing MDA level and inducing both SOD and GSH enzymes, besides it inhibits these enzymes from leaking through cellular membranes so that it was considered as a powerful ROS scavenger. Other scientists investigated the role of Propolis in decreasing nitric oxide and hydrogen peroxide levels that performance an important role in cellular death. They further claimed that additional substances, such as caffeic acid phenethyl ester (CAPE), which prevents the production of reactive oxygen species (ROS) in numerous systems, might be granted the antioxidant effect of Propolis **(16, 38, 39).**

Formerly some researchers established that Propolis modulates the process of fibrosis induced by TGF-1 cytokine **(40).** Propolis stops apoptosis and processing of cell death by inhibiting nitric oxide synthase, cytochrome C and caspase-3 productions. Many researchers documented the same consequences and demanded this activity to as a component of Propolis **(13).**

Conclusion

Propolis is a natural product with anti-inflammatory, antioxidant and anti-cytotoxic activities that have a fundamental protective role against the harmful exposure to Chromium in lung tissue.

References:

1. Scarselli A, Binazzi A, Marzio DD, Marinaccio A, Iavicoli S. (2012): Hexavalent chromium compounds in the workplace: assessing the extent and magnitude of occupational exposure in Italy. J Occup. Environ Hyg. 2012; **9**(6):398–407. Doi: 10.1080/15459624.2012.682216.

2. Caglieri A, Goldoni M, Acampa O, Andreoli R, Vettori MV, Corradi M, et al. (2006): The effect of inhaled chromium on different exhaled breath condensate biomarkers among chrome-plating workers. Environ Health Perspect. 2006; **114**(4):542–6. Doi: 10.1289/ehp.8506

3. Gatto NM, Kelsh MA, Mai DH, Suh M, Proctor DM. (2010): Occupational exposure to hexavalent chromium and cancers of the gastrointestinal tract: a meta-analysis. Cancer Epidemiology, 2010; **34**(4):388–99. Doi: 10.1016/j.canep.2010.03.013.

4. Seidler A, Jahnichen S, Hegewald J, Fishta A, Krug O, Ruter L, et al. (2013): Systematic review, and quantification of respiratory cancer risk for occupational exposure to hexavalent chromium. Int Arch Occup Environ Health. 2013; 86(8):943–55. Doi: 10.1007/s00420-012-0822-0.

5. Li Chen T, LaCerte C, Wise SS, Holmes A, Martino J, Wise JJ, et al. (2012): Comparative cytotoxicity and genotoxicity of particulate and soluble hexavalent chromium in human and sperm whale (Physeter macrocephalus) skin cells. Comp. Biochem. Physiology C Toxicol Pharmacology. 2012; **155**(1):143–50. Doi: 10.1016/j.cbpc.2011.03.011.

6. Kerger BD, Paustenbach DJ, Corbett GE, Finley BL. (1996): Absorption and elimination of trivalent and hexavalent chromium in humans following ingestion of a bolus dose in drinking water. Toxicol Appl Pharmacol. 1996; **141**(1):145–58. Doi: 10.1006/taap.1996.0271.

7. Kumar D, Gangwar SP. (2012): Role of antioxidants in detoxification of Cr (VI) toxicity in laboratory rats. J Environ Sci Eng. 2012; **54**(3):441–6. https://pubmed.ncbi.nlm.nih.gov/24749208/

8. Xiao F, Li Y, Dai L, Deng Y, Zou Y, Li P, et al. (2012): Hexavalent chromium targets mitochondrial respiratory chain complex I to induce reactive oxygen species-dependent caspase-3 activation in L-02 hepatocytes. Int J Mol Med. 2012; **30**(3):629–35. Doi: 10.3892/ijmm.2012.1031.

9. Adams RJ, Wilson DH, Taylor AW, Daly A, Tursan d'Espaignet E, Dal Grande E, et al. (2006): Coexistent chronic conditions and asthma quality of life: a population-based study. Chest. 2006;**129**(2):285–91. Doi: 10.1378/chest.129.2.285.

10. Antonini JM, Roberts JR, Schwegler-Berry D, Mercer RR. (2013): Comparative microscopic study of human and rat lungs after overexposure to welding fume. Ann Occup Hyg. 2013; **57**(9):1167–79. Doi: 10.1093/annhyg/met032.

11. Asatiani N, Kartvelishvili T, Abuladze M, Asanishvili L, Sapojnikova N. (2011): Chromium (VI) can activate and impair antioxidant defense system. Biol Trace Elem Res. 2011; **142**(3):388–97. Doi: 10.1007/s12011-010-8806-y.

12. Samuel JB, Stanley JA, Vengatesh G, Princess RA, Muthusami S, Roopha DP, et al. (2012): Ameliorative effect of vitamin C on hexavalent chromium induced delay in sexual maturation and oxidative stress in developing Wistar rat ovary and uterus. Toxicol Ind Health. 2012 ; **28**(8):720–33.Doi: 10.1177/0748233711422728.

13. Oladayo MI. (2016): Nigerian Propolis improves blood glucose, glycated hemoglobin A1c, very low-density lipoprotein, and high-density lipoprotein levels in rat models of diabetes. Journal of Intercultural Ethnopharmacology; 5(3):233. Doi.org/10.5455/jice.20160502065029

14. **Araujo, M. A., Libério, S. A., Guerra, R. N., Ribeiro, M. N. S., and Nascimento, F. R. (2012).** Mechanisms of action underlying the anti-inflammatory and immunomodulatory effects of propolis: a brief review. Revista Brasileira de Farmacognosia, 22, 208-219. https://Doi.org/10.1590/s0102-695x2011005000167

15. **Sabir, A. (2016):** The effect of propolis on cytokines during dental pulp inflammation. Journal of Apiculture, 31(1), 97-101. https://Doi.org/10.17519/apiculture.2016.04.31.1.97

16. **Mai F Tolba, Samar S Azab, Amani E Khalifa, Sherif Z Abdel-Rahman, Ashraf B Abdel-Naim (2013):** Caffeic acid phenethyl ester, a promising component of propolis with a plethora of biological activities: a review on its anti-inflammatory, neuroprotective, hepatoprotective, and cardioprotective effects. IUBMB Life, 2013 Aug; 65(8):699-709, Doi: 10.1002/iub.1189.

17. **Türkez H., Yousef M.I. and Geyikoglu F. (2010):** Propolis prevents aluminum-induced genetic and hepatic damage in rat liver. Food and Chemical Toxicology, 48(10):2741–2746. Doi.org/10.1016/j.fct.2010.06.049

18. **Calvin J. Kouokam, Idoia Meaza, John Pierce Wise Sr (2021):** Inflammatory effects of hexavalent chromium in the lung: A comprehensive review. Toxicol Appl Pharmacol, 2022 Nov 15:455:116265. Doi: 10.1016/j.taap.2022.116265.
19. **Soudani N, Rafrafi M, Ben Amara I, Hakim A, Troudi A, Zeghal KM, Ben Salah H, Boudawara T, Zeghal N. (2013):** Oxidative stress-related lung dysfunction by chromium (VI): alleviation by Citrus aurantium L. Physiol. Biochem. 69(2):239-53 (2013). Doi: 10.1007/s13105-012-0207-6.
20. **Bancroft, J. and Gamble, M. (2008):** Theory and Practice of Histological Techniques; 6th ed: 165-175.
21. **Takahashi, S., Shinzato, K., Domon, T., Yamamoto, T., and Wakita, M. (2004):** Mitotic proliferation of myoepithelial cells during regeneration of atrophied rat submandibular glands after duct ligation. Journal of oral pathology & medicine, 33(7), 430-434. Doi: 10.1111/j.1600-0714.2004.00234.x.
22. **Quinteros FA, Poliandri AH, Machiavelli LI, Cabilla JP, Duvilanski BH. (2007):** In vivo and in vitro effects of chromium VI on anterior pituitary hormone release and cell viability. Toxicol Appl Pharmacol. 2007;218 (1):79–87, Doi: 10.1016/j.taap.2006.10.017.
23. **Navya, K., & Phani Kumar, G., Chandrasekhar, Y., & Anilakumar, K. R. (2018).** Evaluation of potassium dichromate (K2Cr2O7)-induced liver oxidative stress and ameliorative effect of Picrorhiza kurroa extract in wistar albino rats. Biological Trace Element Research, 184(1), 154-164. Doi: 10.1007/s12011-017-1172-2.
24. **Boşgelmez, I. I., and Güvendik, G. (2004):** Effects of taurine on oxidative stress parameters and chromium levels altered by acute hexavalent chromium exposure in mice kidney tissue. Biological Trace Element Research, 102(1-3), 209-225. Doi: 10.1385/BTER:102:1-3:209.
25. **Ambreen, K., Khan, F. H., Bhadauria, S., & Kumar, S. (2014): Genotoxicity** and oxidative stress in chromium-exposed tannery workers in North India. Toxicology and Industrial Health, 30(5), 405-414. Doi: 10.1177/ 0748233712457447.
26. **Iztleuov M, Zhexenova A, Abdildayeva S, Iztleuova G, Zhiengalieva A, Altayeva A, Taskozhina G, Jumagalieva J. (2017):** Influence of Boron Compounds on Chromium-Induced Hemorheology Disorders in Rats. Biomedical & Pharmacology Journal. 10(4):1779-1786 (2017) Doi: 13005/bpj/1292 pathology1.
27. **Bosgelmez, I.I. and Güvendik G. (2019):** Beneficial effects of N-acetyl-L-cysteine or taurine pre-or post-treatments in the heart, spleen, lung, and testis of hexavalent chromium-exposed mice, Biol. Trace Elem. Res. 190 (2) (2019) 437–445, Doi.org/10.1007/s12011-018-1571-z
28. **Marat I., Abdiraman K., Aigul T.,Dariga Y., Ainur A., Assem T., Yerbolat I. and Gulmira I. (2020):** The Effect of Sodium Tetraborate on Chromium-Induced Oxidative Damages in Rats Lung Tissue. Biomed. & Pharmacol. J, Vol. 13(1), 281-290 (2020), Doi.org/10.13005/bpj/1887
29. **Velma, V.; Tchounwou, P.B. (2010):** Chromium-Induced Biochemical, Genotoxic and Histopathologic Effects in Liver and Kidney of Goldfish, Carassius Auratus. Mutat. Res. 2010, 698, 43–51, Doi: 10.1016/j.mrgentox.2010.03.014.
30. **Wang, X.; Son, Y.O.; Chang, Q.; Sun, L.; Hitron, J.A.; Budhraja, A.; Zhang, Z.; Ke, Z.; Chen, F.; Luo, J.; et al. (2011):** NADPH Oxidase Activation Is Required in Reactive Oxygen Species Generation and Cell Transformation Induced by Hexavalent Chromium. Toxicol. Sci. 2011, 123, 399–410, Doi: 10.1093/toxsci/kfr180.
31. **Y. Lv, H. Jiang, S. Li, B. Han, Y. Liu, D. Yang, J. Li, Q. Yang, P. Wu, Z. Zhang. (2020):** Sulforaphane prevents chromium-induced lung injury in rats via activation of the Akt/GSK-3β/Fyn pathway, Environ. Pollut. 259 (2020), 113812, Doi: 10.1016/j.envpol.2019.113812.
32. **Schneider BC, Constant SL, Patierno SR, Jurjus RA, Ceryak SM. (2012**): Exposure to particulate hexavalent chromium exacerbates allergic asthma pathology. Toxicology and applied pharmacology. 2012; 259 (1):38±44. Doi: 10.1016/j.taap.2011.12.001.
33. **X.F. Wang, X.M. Lou, Y. Shen, M.L. Xing, L.H. Xu. (2010):** Apoptotic-related protein changes induced by hexavalent chromium in mice liver, Environ. Toxicol: Int. J. 25 (1) (2010) 77–82, Doi: 10.1002/tox.20478.
34. **Y.H. Wu, J.C. Lin, T.Y. Wang, T.J. Lin, M.C. Yen, Y.H. Liu, P.L. Wu, F.W. Chen, Y. L. Shih, I. Yeh. (2020):** Hexavalent chromium intoxication induces intrinsic and extrinsic apoptosis in human renal cells, Mol. Med. Rep. 21 (2) (2020) 851–857, Doi: 10.3892/mmr.2019.10885.
35. **Zhang Y., Y. Ma, N. Liang, Y. Liang, C. Lu, F. Xiao. (2019):** Blockage of ROS-ERK-DLP1 signaling and mitochondrial fission alleviates Cr (VI)-induced mitochondrial dysfunction in L02 hepatocytes, Ecotoxicol. Environ. Saf. 186 (2019), 109749, Doi: 10.1016/j.ecoenv.2019.109749.
36. **Eman S El-Roghy, Maha El-Sayed soliman, Samy Atteya, Hend Zakaria and Seham Ahmed Mohammed Abdel Aziz (2021):** Evaluation of Propolis Supplementation on Lung Tissue Toxicity Induced by Aluminum chloride in Adult Male Albino Rats: A Histological and Immunohistochemical Study. E.J.H. Vol. 45, No. 4, 1110-0559. Doi: 10.21608/ejh.2021.84076.1519.
37. **Korish, A. A. and Arafa, M. M. (2011).** Propolis derivatives inhibit the systemic inflammatory response and protect hepatic and neuronal cells in acute septic shock. The Brazilian Journal of Infectious Diseases, 15(4), 332-338, https://pubmed.ncbi.nlm.nih.gov/21861003/
38. **Mounieb F, Ramadan L, Akool ES, Balah A (2017):** Propolis alleviates concanavalin A-induced hepatitis by modulating cytokine secretion and inhibition of reactive oxygen species. Naunyn Schmiedebergs Arch Pharmacol 390: 1105-1115, 2017, Doi: 10.1007/s00210-017-1410-3.
39. **Sforcin, J. M. (2016):** Biological properties and therapeutic applications of propolis. Phytotherapy research, 30(6), 894-905, Doi: 10.1002/ptr.5605.
40. **de Moura SA, Ferreira MA, Andrade SP, Reis ML, Noviello Mde L and Cara DC. (2011):** Brazilian green Propolis inhibits inflammatory angiogenesis ina murine sponge model. Evid Based Complement Alternat Med. 2011; 2011:182703, Doi: 10.1093/ecam/nep197.