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Anti-inflammatory and antioxidant effects of zinc and vitamin D on nicotine-induced oxidative stress in adult male rats

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***Abstract*---**Current study aimed to investigation the protective effects of zinc and vitamin D in attenuating alveolar response, as anti-inflammatory and antioxidant, in nicotine stressed male rats. Thirty adult males Wistar rats, as a model of mammals, randomly assigned to five equal groups and treatment as follows for 14 days: Control (C) drenched vehicle without treatment, G1 = injected nicotine 1.5 mg/ kg b.w. i/P, G2 administrated orally of zinc 60 mg/ kg b.w., G3 = administrated orally of vitamin D 250 µg/ kg b.w. and G4 administrated orally both of zinc and vitamin D with same doses and stressed by nicotine 1.5 mg/ kg b.w. i/P. At the end of the experiment, rats were anesthetized blood samples have been collected, and then iron, cytokines (IL-6) and malondialdehyde (MDA) and glutathione peroxidase (GPX) were estimation in in serum and lung tissues. The result showed significant ($p \leq 0.5$) increase in serum MDA in group G1 when compared with other groups. While significant ($p \leq 0.5$) increase in serum GPx in all treatment group in comparison with nicotine treated group and control. Besides, administration of zinc and vitamin D showed highly effective decrease in alveolar MDA in G2 and G3 groups when compared with other treatment group and control. On the other hand, nicotine-stressed rats treated with zinc and vitamin D (G4) shows a significant decreased in lung tissue MDA when compared with other groups. All treatment groups were manifested by highly significant increase of lung tissue GPx when compared to G1 and control. The results are also shown significant increase of serum iron and pro-inflammatory cytokine IL-6 in nicotine treated group comparing to other groups. Furthermore, administration of zinc and

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vitamin D cause ameliorating of theses parameters in nicotine-stressed rats (G4).In conclusion, these results referred to the ability of zinc and vitamin D as antioxidants, immunomodulatory and protective effects against oxidative stress induced by nicotine in rats.

***Keyword*---**Nicotine, zinc, vitamin D, MDA, GPx, iron and IL-6**.**

Introduction

Nicotine is the active chemical in tobacco, and tar and nicotine are the main components of tobacco smoke (Ateyya et al. 2016). Nicotine is a severe risk factor for lung and cardiovascular disease, as well as other ailments, when combined with the tars and carbon monoxide found in cigarette smoke (Zaki 2019). Nicotine and tar, which create flavor, provide immediate gratification when smoking (Kalpana and Menon 2004). Increased reflex air-ways resistance, reduced ciliary activity following transitory stimulation, and carbon monoxide absorption are all acute effects of smoking tobacco, especially in the presence of coronary heart disease (Xiao et al. 2016). Low doses of nicotine cause depolarization of ganglion stimulation, while increased nicotine causes ganglion depression (Whalen 2019). Specific effects of nicotine on the blood-brain barrier are investigated by increased signaling activity associated with expression by endothelial nicotinic acetylcholine receptors and a decrease in tight junction proteins (Hawkins et al. 2004). Nicotine stimulates the release of catecholamine, serotonin, antidiuretic hormone, corticotrophin, and growth hormone in the CNS (Lambers and Clark 1996). Nicotine has been shown to produce adrenal medulla, a branch of the sympathetic nervous system, and to produce and release norepinephrine / epinephrine (Oakes et al. 2021). Nicotine activates tyrosine hydroxylase (an enzyme that reduces the biosynthetic pathway of catecholamine), dopamine hydroxylase and dopamine hydroxylase that alter adrenal expression (Oakes et al. 2021). Vitamin D inhibits monocyte/macrophage production of pro-inflammatory cytokines like IL-6 or TNF- in monocytes by inhibiting p38 MAP kinase 1.25(OH)2 D3 induces MAPK phosphatase-1 (MKP-1) which can dephosphorylate p38 and inactivate p38 MAP kinase, inhibiting gene expression and protein release of pro-inflammatory mediators in monocytes (Zhang et al. 2012; Wöbke et al. 2014; Adeeb et al. 2017).Cytokine storm has received a great deal of attention in connection with the pathology of corona virus disease 2019 (COVID-19) (Mahmudpour et al. 2020; Hu et al. 2021).IL-6 is a prototypical member of the IL-6 family of cytokines(Hunter and Jones 2015).Interleukin 6 (IL-6) is a predictive factor of poor prognosis in patients with acute respiratory distress syndrome (ARDS) and it is have been identified as early biomarkers of lung injury (Vaporidi et al. 2008).

Zinc reduces production of tumour necrosis factors (TNF-), interferon-c (IFN-c), and IL-2, but has no effect on Th2 cells (IL-4, IL-6, and IL-10) (Olechnowicz et al. 2018). To identify the signaling pathways involved in zinc-mediated alterations in the actin cytoskeleton, researchers used hypoxia-released zinc to cause contraction in isolated pulmonary endothelial cells. hypoxia-induced phosphorylation of the contractile protein myosin light chain (MLC) and actin stress fiber construction (Bernal et al. 2008; Slepchenko et al. 2016). Although

Zn_2^+ has been shown to prevent apoptosis at low concentrations,, while large amounts of Zn_2^+ cause apoptosis or necrosis to cause death among a variety types (Bozym et al. 2010). Apoptosis is suppressed by two major mechanisms: one produces a thiolate complex reduced from the sulfhydryl group of the Zn^{2+} protein and reduces the oxidation of the protein by ROS. Second, Zn^{2+}-induced inhibition of caspase-3 activation (Bossy-Wetzel et al. 2004; Al-ghareebaw and Al-Okaily 2020). According to other research, Zn_2^+ exposure raised Bax levels increases mitochondrial transition pore permeability, reduce mitochondrial transmembrane potential, and reduces Bcl-2 and surviving expression levels, all of which led to cell death (Ku et al. 2012).

Reduced G6PD activity inhibits the cell's ability to produce NADPH, which is required to maintain a reduced glutathione pool. As a result, cell depletion of free radicals and peroxides produced by the cells is reduced. Glutathione also preserves the reduced sulfhydryl groups of proteins such as hemoglobin (Murray et al. 2003). Sulfhydryl groups oxidize and cause protein transport to form insoluble substances (called Heinz's) that bind to the membrane of red blood cells (Whalen 2019). Excessive oxidation of membrane proteins strengthens red blood cells (does little damage) and macrophages in the spleen and liver remove them from circulation. G6PD deficiency affects all cells in the body; it is most severe in erythrocytes, where the pentose phosphate pathway is the only way to produce NADPH (Harvey 2011).

Materials and Method

Nicotine 72290 (-) nicotine (-)- nicotine >97%(GC); KP 243-248° $C10H_{14}N_2$ Mr. 162.24..Switzerland . Vitamin D Switzerland –acino and zinc

Experimental design

Thirty (30) adult male rats were kept at (23±2C°) have been at random divided into five groupsequally of experiment and treatment for 14 days were maintained at room temperature Rats. Control group: administered orally and injected with sterile distilled water, G1: injected with nicotine 1.5mg/kg b.w. I.P., G2 administered 60 mg/kg.b.w. of zinc orally, G3: administered 250 µg /kgb.w. of vitamin D orally and G4: administered both zinc and vitamin D with same doses orally and injected with nicotine 1.5mg/kg. I.P at 14 days. Rats were anesthetized with ketamine at the end of the treatment period. (kitamine 100 mg/ kg I.P) with xylazine (10mg/kg I.P) **(Veilleux-Lemieux et al. 2013),** and blood samples have been collected from the optical vein, then animals were sacrificed and lung was removed. Blood samples have been use for serum iron, IL-6, MDA and GPx of serum and lung tissue assessment.

ELISA kit

Cat. Number: MBS3808464iron Rat ELISA kitand rat IL-6 catalog Number: MBS269892.Rats serumIL-6 andiron concentration was measured using ELISA technique.

Malondialdehyde (MDA) Determination: Rats serum and lung tissue Malondialdehyde (MDA) concentration was measured by according to the method of **(Guidet and Shah; 1989)**

Determination of SerumGlutathione peroxidase(GPX): Rats serum glutathione peroxidase (GPx)concentration was measured by according to the method of Paglia and Valentine on the principal of DTNB**(Razygraev et al. 2019)**.

Result

The result of serum MDA concentration clarified in figure (1) showed that mature male Westar rats administered zinc(60 mg/ kg b.w for 14 days) and vitamin D (250 µg/ kg b.w for 14 days) revealed a significant highest increment (P< 0.05) of the nicotine stressed group (G1) when compared with the negative control C. On the other hand, treated groups of zinc (G2) and vitamin D group (G3) recorded insignificant (P< 0.05) elevation when compared with each other and between G2 with negative control, while significant(P< 0.05) decreased between G3 and G4.On the other hand nicotine-stressed rats treated with zinc and vitamin D(G4 that shown highly decreased significant (P < 0.05) when compared with the nicotine group (G1) and insignificant when compared between G4 with the control group. Results of serum GPx level clarified in figure (1) revealed that male rats treated with vitamin D and zinc with oxidative stress induced by nicotine showed high significant increase level of serum GPx in treated groups (G2 and G3) when compared with negative control group C. On the other hand, G4 group registered serum GPx level a highly significant increase (P < 0.05) when compared with nicotine group (G1) and negative control group (C). Nicotine-stressed rats treated with zinc and vitamin D(G4), that recorded highest increment level of serum GPx than that of other treatment groups which showed significances when compared with each other.

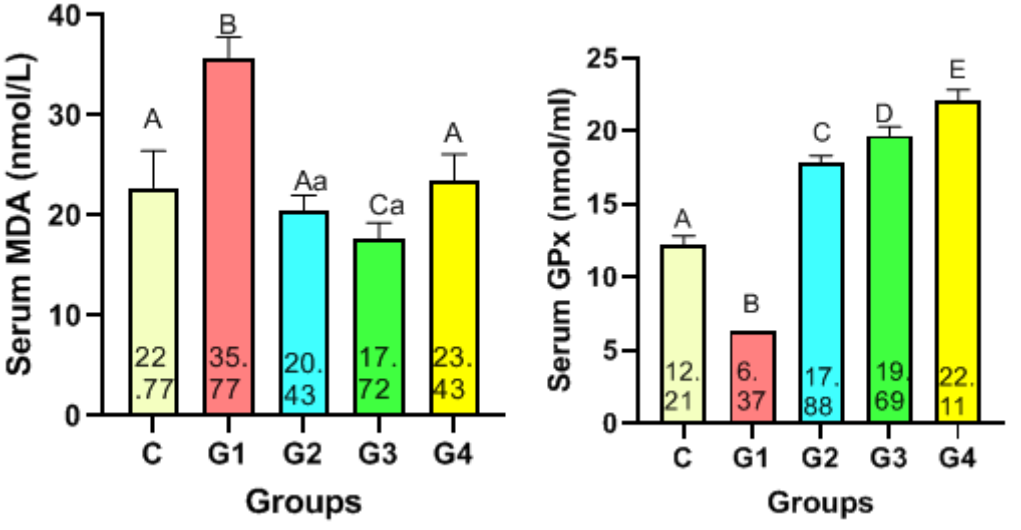

Figure 1 : Effect of nicotine, zinc and vitamin D on serum MDA and GPx in adult male rats after 14 days

C = Control drenched vehicle without treatment for 14 days.
G1 = injected with i/p nicotine 1.5 mg/ kg b.w.I.P.
G2 = administrated with orally of zinc 60 mg/ kg b.w.

G3 = administrated with orally of vitamin D 250 µg/ kg b.w.
G4 = administrated with orally of zinc 60 mg/ kg and vitamin D 250 µg/ kg stressed with nicotine 1.5 mg/ kgb.w.I.P.
Values are expresses as mean ± SD, n=5
Different capital letters mean significantly (p≤ 0.5) different between groups.

Figure (2) demonstrates results of lung tissue MDA concentrations throughout the experimental period. Interestingly, levels of serum MDA in G2 and G3 groups were extremely ($P < 0.05$) highest significant decreased when compered with (G1 and C), and nicotine-stressed rats treated with zinc and vitamin D(G4) roup was decremented ($P < 0.05$) when compared with the nicotine group G1, so insignificant between G4 and the negative control C.

Figure (2), all treatment groups (G2, G3) and (G4) showed a highly significant ($p < 0.05$) increment in lung tissue GPx concentration compared to control and highest significant ($p < 0.05$) increase in nicotine-stressed rats treated with zinc and vitamin D(G4) when compared with positive control nicotine group G1, while there was a highly significant decrement in nicotine group G1 when compared with all treatment groups (G2, G3 and G4) and with the negative control group C.

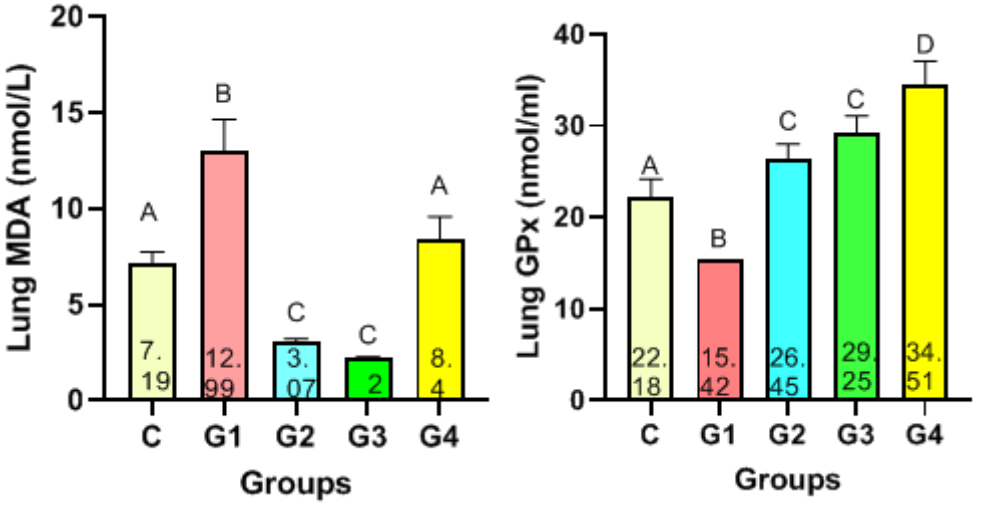

Figure (2): Effect of nicotine, zinc and vitamin D of MDA and GPx of lung tissue in adult male rats after 14 days

C = Control drenched vehicle without treatment for 14 days.
G1 = injected with i/p nicotine 1.5 mg/ kg b.w.I.P.
G2 = administrated with orally of zinc 60 mg/ kg b.w.
G3 = administrated with orally of vitamin D 250 µg/ kg b.w.
G4 = administrated with orally of zinc 60 mg/ kg and vitamin D 250 µg/ kg b. w. stressed with nicotine 1.5 mg/ kgb.w. I.P.
Values are expresses as mean ± SD, n=5
Different capital letters mean significantly (p≤ 0.5) different between groups.

This study aimed to evaluate the impact of zinc and vitamin D in attenuating nicotine -induced oxidative stress on certain parameters related to the lung in

adult male rats. The animals used in this experiment did not show any abnormalities or health deviations. This indicates that the safety of daily administration of 1.5mg/kg of nicotine intraperitoneally concurrently with oral administration of zinc 60 mg/kg and vitamin D 250 µg/kg for 14 days of experiments .

Current results showed inordinate ROS production in the nicotine-treated group causes an increase in MDA levels in the lungs, which might also represent a failure of the lungs for ROS elimination with downregulation of antioxidants produced due to excessive production of oxidants. MDA could be used as an oxidative stress indicator and can alter membrane permeability (Al-Okialy, 2018; Oakes et al. 2021).

As previously reported by Hamza and El-shenawy (2017) the results showed that intraperitoneal nicotine injection induced a significant increase in serum MDA concentrations while decreasing GPx activity compared to the control group, giving the impression of induced oxidative stress. This result is in agreement with (Budzynska et al. 2013; Chattopadhyay 2016; Hamza and El-shenawy 2017; Schweitzer et al. 2021). Oxidative stress is characterized by the lower activity of antioxidant enzymes such as GPx. Antioxidant ability may be impaired with the presence of ROS, resulting in variable damage to DNA, protein, and fatty acids. Enhanced ROS has resulted an increase in levels of MDA. A number of reports have shown that the administration of nicotine increases ROS generation within the lungs(Ahmad, et al. 2019; Schweitzer et al. 2021).

In rat lung cells, lipid peroxidation was elevated while antioxidant GPx levels were reduced. Nicotine produces ROS, which causes an increase in MDA in the lungs of mice. Another previous study found that oxidative stress was the mechanism responsible for nicotine-induced toxicity in lung tissue (Dhouib et al., 2015; Hamza and El-Shenawy 2017; Oakes et al. 2021). In addition, current studies have shown that the administration of nicotine to rats increased GPx levels in lung tissue. Cells use a variety of antioxidant mechanisms to reduce the levels of excess ROS (Al-Okialy, 2018). Increased oxidative stress could indeed explain the observed decrease in anti-oxidative enzyme activity in the lung as GPx, which suggested scavenging of free radicals from nicotine-exposed lung tissues. GPx is a critical stimulator of the anti-oxidative defense system and the preservation of cellular redox potential (Leopold and Loscalzo 2005; Ahmad, et al. 2019; liu, et al., 2022).

results of histopathological examination revealed that in the second week of treatment with nicotine (1.5 mg/kg/day) injections, thickening of inter-alveolar septa caused extensive destruction of the alveolar wall and the formation of enlarged, irregular airspace. As a result of nicotine, changes in lung architecture, inflammatory infiltrations, abnormal air spaces, and a rise in alveolar septal thickening were dominant in lung tissue, and these findings are consistent with previous research (El-Sokkary et al. 2007; Valado et al. 2022).
Histopathological findings support biochemical mutations by identifying significant morphological changes in the lungs of nicotine-treated mice. This could be due to the release of TNF-α, different types of ILs, and free radicals, which can damage the alveolar structure and cause lung congestion (Ateyya et al.

2016; Oakes et al. 2021). The presence of neutrophils in saliva has been shown to be directly associated with pulmonary dysfunction, suggesting a strong association between neutrophil inflammation and airway obstruction (Dhouib et al. 2015).

In the present study, treatment groups of adult male rats with zinc 60 mg/kg and vitamin D 250 µg/kg, stressed by nicotine1.5 mg/kg caused regularization of oxidative/antioxidant parameters in the lungs in MDA level of serum and lung tissue, whereas highly significant increment in serum GPx and lung tissue, this results due to modulation of zinc and vitamin D effects on reduction of free radicals and oxidative stress, which liberation by nicotine effects (Mohamed and Abdelrahman 2019; Mousavi et al. 2020). In our study, zinc showed antioxidant capacity by lowering the level of MDA in the lungs of the nicotine group compared to the nicotine group (Mohamed and Abdelrahman2019). Note that Vitamin D has a strong antioxidant effect, reduces oxidative stress and inhibits free radicals, especially MDA products. Current results are consistent with previous studies showing that low serum MDA levels in product animals given 60 mg/kg of zinc. Previous studies have shown that zinc and vitamin D reduce MDA levels and oxidative stress by increasing GPx levels (Hajiluian et al., 2017). In contrast, our data provide the first study on the effects of zinc and vitamin D on nicotine toxicity in lung tissue. In addition, vitamin D supports cellular oxidation and reduction (redox) control by maintaining normal mitochondrial functions (63) through downregulatingthe intracellular oxidative stress-related activities, when the level of vitamin D is adequate (Bouillon and Verstuyf 2013 and Ryan et al. 2016).Also, the intracellular Nrf2 level is inversely correlated with the increase of mitochondrial ROS (Tseng, et al. 2013)and the consequent generation of oxidative stress. So, Nrf2 plays a key role in protecting cells against oxidative stress; this is modulated by enhance the expression of Nrf2 by vitamin D (Tullet et al. 2017).

The result of serum iron in the figure (3) significant ($p < 0.05$) decrease in serum iron in groups (G2 and G3) comparing to control group and insignificant between each other. Whereas nicotine-stressed rats treated with zinc and vitamin D(G4) howed significant ($p < 0.05$) decreased in serum iron when compared to (nicotine group) G1, due to antioxidant effect of zinc and vitamin D was counteract the oxidative stress of nicotine.

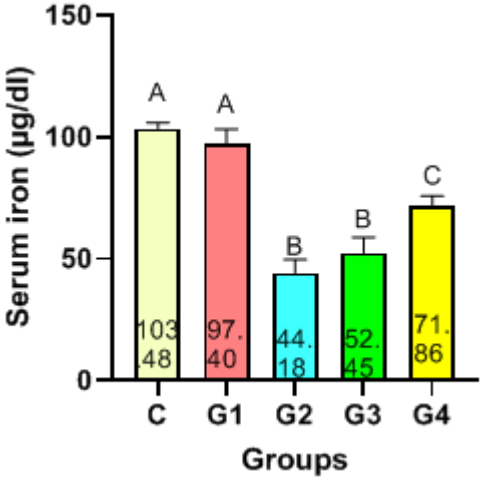

Figure (3): Effect of nicotine, zinc and vitamin D on serum iron in adult male rats after 14 days

C = Control drenched vehicle without treatment for 14 days.
G1 = injected with i/p nicotine 1.5 mg/ kg b.w.I.P.
G2 = administrated with orally of zinc 60 mg/ kg b.w.
G3 = administrated with orally of vitamin D 250 µg/ kg b.w.
G4 = administrated with orally of zinc 60 mg/ kg and vitamin D 250 µg/ kg b. w. stressed with nicotine 1.5 mg/ kgb.w.I.P.
Values are expresses as mean ± SD, n=5
Different capital letters mean significantly (p≤ 0.5) different between groups.

The current study's findings further indicated that nicotine's adverse effect on pulmonary function may be due to decreased serum iron concentrations. Other research has confirmed this (Zhang et al. 2019). In contrast to the limited availability of iron, free "labile," redox active ferrous iron is prone to creating highly reactive OH-radicals by reacting with H2O2 in the Fenton reaction, eventually leading to oxidative cell injury. Intracellular iron is thus divided into different "cellular labile iron pools" that communicate via protein-based iron shuttles (Bresgen and Eckl 2015). As previously noted, ferritin-based iron buffering is critical for cellular integrity, especially in the face of increased oxidative stress. Despite the fact that ferritin has a maximum storage capacity of 4500 Fe3+ ions per molecule, (Arosio etal. 2008). In the same manner during the respiratory burst, the effect of nicotine on male rats' lung tissue causes them to produce hypochlorous acid (HClO), which reacts with unsaturated fatty acids, proteins, and any oxidizable group, causing protein adducts and genetic mutations, as well as influencing signaling pathways. This is caused by a decrease in serum iron (Chattopadhyay 2016; Hamza and El-shenawy 2017). Ferritin reduces oxidative stress very quickly by catalytic release of O2 or reactive oxygen species (these are made from Fe3 + Fe2 + substrate and release Fe2 + ions or iron-producing enzymes such as catalase. Oxidative stress is associated with high H2O2 imbalances, but the effect of reducing oxidative stress is clear (Bradley et al. 2016). Generally, it is not always been proven whether the upregulated ferritin reduces oxidative stress by catalyzing the removal of O2 or reactive oxygen

species as Fe3+ is produced from a Fe2+ substrate, emptying Fe2+ ions, or causing an increase of iron for the production of enzymes like catalase, which directly decreases oxidative stress by massive imbalance of H2O2, but the function in decreasing oxidative stress is evident (Bradley et al. 2016; Al-Abedi et al., 2020). Recycling iron from erythrocyte phagocytoses by the macrophages plays an important role in upregulation and systemic iron storage,with high iron content, macrophages produce more ferritin and actively excrete it into the circulation. Since macrophages are a major source of serum ferritin and serum ferritin levels indicate iron stores in the body, serum ferritin is clinically used as a reliable indicator of systemic iron status (Lee, et al. 2016; Fabiano et al. 2018; Hiroshi Kawabata, 2018). It is well known that macrophages perform different biological functions, mainly clearance of pathogens, apoptotic and senescent cells, in this context Recalcati and Cairo, (2021) referred the major targets of homeostatic phagocytosis by macrophages are old/damaged red blood cells, besides, it seem particularly adapted to store amounts of iron that may be toxic to other cells. On the other hand, in line with this purpose, Vitamin D may also influence circulating iron status by stimulating erythropoiesis and suppressing hepcidin expression. Low levels of pro-inflammatory cytokines and hepsidin increase serum iron biology from erythropoiesis and hemoglobin synthesis by inhibiting iron clearance in macrophages (Masoud et al. 2018). Our findings suggest that physiologic doses of chronic oral zinc supplementation inhibit the absorption of iron in the male rats. The adverse effects of zinc on serum iron do not exacerbate anemia, the duration and dose of supplementation should be considered (de Brito et al. 2014).

Zinc and iron can bind due to the chemical similarities between the two trace elements. Therefore, the inhibitory effect of zinc on iron absorption (and vice versa) may be related to zinc antagonistic to iron absorption in the intestinal tract (Rolf et al., 2021). The absorption and transport mechanisms of zinc and iron are chemically similar. The quantitative effect of this relationship is tested by the amount of food concentrate used and the amount of zinc and iron when using aqueous zinc and iron solutions, foods, and in some cases, supplements. They have antagonist effects (Olivares et al., 2012; de Brito et al., 2014). High dosages of zinc in aqueous solutions have been shown to impair iron absorption, whereas zinc added to meals has had no effect (Olivares et al. 2012). This is in contrast to our findings, which showed that supplementation of zinc orally can lower serum iron levels in healthy school children for 90-day zinc supplement (Jayalakshmi and Platel 2016). It demonstrates the results of serum IL-6, (G1) nicotine group registered significantly($P< 0.05$) increased concentration level of IL-6 among all treatment groups, while significant ($P< 0.05$) decrement of nicotine stressed rats treated with zinc and vitamin D group G4 compared with positive control (nicotine group) G1, as well as insignificant between G4 and negative control group C. On the other hand, statistical analysis revealed significant ($P< 0.05$) increased of treated groups zinc G2 and vitamin D G3 when compared with C

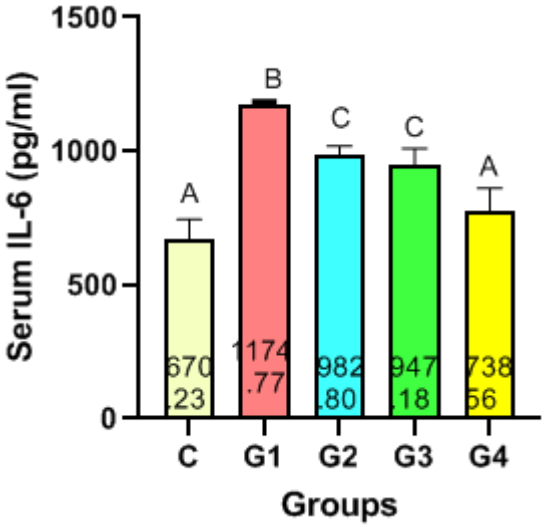

Figure (4): Effect of nicotine, zinc and vitamin D on serum IL-6 in adult male rats after 14 days

C = Control drenched vehicle without treatment for 14 days.
G1 = injected with i/p nicotine 1.5 mg/ kg b.w.I.P.
G2 = administrated with orally of zinc 60 mg/ kg b.w.
G3 = administrated with orally of vitamin D 250 µg/ kg b.w.
G4 = administrated with orally of zinc 60 mg/ kg and vitamin D 250 µg/ kg b. w. stressed with nicotine 1.5 mg/ kgb.w.I.P.
Values are expresses as mean ± SD, n=5
Different capital letters mean significantly (p≤ 0.5) different between groups.

In the present work, we have found that stressed male rats, induced by nicotine cause an increase in serum IL-6 concentration. This result was agreement with Ung et al., (2019) who found thatnicotine stimulated IL-6 expression via the activator protein 1 (AP-1) transcription factor (Ung et al. 2019). Also, our results was accordance with other researches which reported that nicotine activated a mechanism that produced high levels of pro-inflammatory cytokines, particularly IL-6, whereas monocytes and macrophages were activated to remove dead cells and promote tissue repair (Hamza and El-shenawy 2017 and Al-tameemi et al. 2022). This increment in cytokines and adherence molecules causes neutrophils and macrophages to be activated in the lung, which can result in tissue destruction (Ahmad et al. 2019). Among nicotine impacts, they are thought to be serious in causing severe lung injury caused by an increase in IL-6 levels (Wang et al. 2012; Ahmad et al. 2019). Cytokines function in many fundamental processes for life and disease including immunity, inflammation, embryonic development, regeneration, angiogenesis, metabolism, obesity, aging and so on. Among these diverse functions, their roles in inflammation have attracted attention in relation to disease development and treatment (Hirano 2021). Pharmacological inhibition and mutagenesis studies indicated that p38 mitogen-activated protein kinase (MAPK) mediated the IL-6-induced upregulation of nicotine in EA.hy926 cells (Ung et al. 2019). A long-term elevation of IL-6 that could be responsible for severe lung damage and is considered to contribute to

the initiation and extension of the inflammatory process as well as it has ability to inhibit the production of alpha-tumor necrosis factor (α-TNF)(Li et al. 2021).

Zinc is one of the essential trace element that is necessary for the development and growth at all stages of organisms and plays an important role as coenzyme in many reactions (Rolf et al. 2021). A long-term elevation of IL-6 is thought to be responsible for severe lung damage to the immune system and macrophage excitation syndrome. Zinc supplementation inhibits the activation of several pro-inflammatory signaling pathways, including STAT-3 with IL-6, as an anti-inflammatory element (Mayor-Ibarguren, et. al., 2020). Zinc impulses are required for IL-6-increased STAT3 phosphorylation, which has been shown to be enhanced by zinc deficiency. IL-6 mediates B cell stimulation and recogintion into plasma cells, and IL-6 oversupply is linked to autoantibody production. These findings suggest that strict regulation and proper zinc homeostasis are required to keep the immune system balanced (Maywald et al. 2017). Pro-inflammatory and regulatory mechanisms involve zinc. Regardless, it appears clear that low or high zinc levels can cause adaptive and innate immune system dysfunction. Zinc regulates lymphocyte and other leukocyte proliferation, differentiation, maturation, and function. So it modulates the immune response, and a lack of it makes people more susceptible to inflammatory and infectious diseases like pneumonia (Mayor-Ibarguren et al. 2020).

During the experiment, oral administration of vitamin D 250 µg/kg b.w for 14 days caused a decrement in IL-6 level in male rats that were stressed by intraperitoneal nicotine 1.5 mg/kg BW for 14 days. This result refers to the modulating and down regulating of vitamin D on homeostasis of pro-inflammatory immune reaction on alveolar cell function caused by nicotine effects. This lead to an imbalance between adaptive and innate immunity, that caused alveolar cell damage. This result agreement with Silberstein (2021).

The physiological activity of Vitamin D plays an important role in lung health in protecting against oxidative stress caused by nicotine (Zheng et al. 2020). The regulation of lung function by vitamin D has an effect on the status of adaptive and innate immunity by modulating and decreasing the level of IL-6 that is upregulated. With a favorable association between IL-6 and ACE2 levels, angiotensin II receptor presumably upregulates pulmonary epithelial cell ACE2 receptor (Silberstein 2021). Vitamin D's ability to reduce cytokine production, like IL-6, IFN- γ, and IL-12 are contingent on many factors, as it also reduces other signal transduction, including those including nuclear factor kappa-B (NFB) and mitogen-activated protein kinase (MAPK) (Xie et al. 2017;Giménez et al. 2020; Orrù et al. 2020). The level of NF-κB activity in airway epithelial cells corresponds directly with the resolution of infection at 24 h. Increased NF-κB activation improves bacterial clearance, whereas inhibition of NF-κB activity hinders host defense. The critical downstream event regulated by NF-κB activation in airway epithelial cells appears to be CXC chemokine-mediated neutrophil recruitment into the lungs (Chen et al. 2008).

Recently, Silberstein, (2021) revealed that Vitamin D may have advantages over a therapeutic agents such as tocilizumab, as an IL-6 immunomodulator, and, given that it is safe if administered under clinical supervision, there is a strong

rationale for its use. Vitamin D supplementation increased the expression of the gene G6PD, which codes for glucose-6-phosphate dehydrogenase, a rate-limiting step in the creation of NADPH that is required for the function of important antioxidant enzymes such as those associated with the production of reduced glutathione (Stanton 2012 and Chitsaz, et al. 2019). In addition, Vitamin D suppressed particulate matter (PM) pollutant exposure stimulated IL-6 production, increased human bronchial epithelial cells (HBEC) expression of the antioxidant pathway gene *G6PD*, increased the ratio of reduced to oxidised glutathione, and in PM-stimulated cultures decreased the formation of 8-isoprostane (Abrahams et al. 2019). Moreover, considering the increasing effect of vitamin D on G6PD serum level, new therapeutic options may be available for patients suffering from G6PD enzyme deficiency. Nonetheless, prescribing vitamin D supplements needs to be researched more in future (Chitsaz et al., 2019). Vitamin D raised the percentage of intracellular reduced to oxidized glutathione, which is associated with its role in enhancing antioxidant pathways. This function of vitamin D is consistent with prior data that it can conserve several types of epithelial cells from oxidative stress (Holick 2017).

Vitamin D has been found to restore nuclear translocation of the ligand-bound glucocorticoid receptor that has been impaired by oxidative stress (Lan et al. 2014). The protective mechanism alveolar cell destruction of vitamin D through suppressed inflammatory, apoptotic response and stimulating granulocytes macrophage colony stimulating factor (Basalamah, et al. 2018). Furthermore, rising G6PD expression levels of antioxidant mechanism genes and the levels of oxidized glutathione suggest that vitamin D can protect the lungs and airways in asthmatic pathology through its anti-inflammatory and antioxidant effects in the face of air pollutant exposure, suggesting that vitamin D can protect the lungs and airways in asthmatic pathology through its anti-inflammatory and antioxidant effects (Stanton 2012and Adam-bonci et al. 2021).

The present study revealed that the zinc (60 mg/kg) group and vitamin D (250 µg/kg) group showed a significant decrease in IL-6 when compared with the stressed group (G1) and a significant increase in nicotine (G1) group concentrations in stressed male rats when compared with control. While drenching stressed male rats with zinc and vitamin D, under nicotine stress revealed a significant decrease in IL-6 concentrations compared with nicotine stressed male rats. Based on the above it can be concluded that the equation between TH1 and TH2 cytokines is impaired, while treatment with zinc and vitamin D led to a quantitative and qualitative equal increase in TH1 and TH2 cytokines. These findings indicate the potential role of zinc and vitamin D as immunomodulative agent, as TH1 and TH2 cytokines decreased significantly, and the balance between them remained constant. Moreover, the inflammatory response's inclination towards cell-mediated or humoral-mediated responses is dependent on the balance of TH1 and TH2 cytokines. As a result, any event that disrupts the TH1/TH2 axis may have an impact on the response's outcome

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