Histopathological And Allergic Study Of Evaporated Essential Oils In Broiler Chicken

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Abstract
This study aimed to investigate a new technique of evaporation of essential oils(EOs) (oregano essential oil(OEO), Lemongrass essential oil(LEO) and bergamot essential oil (BEO).) on 1 immunological parameters interleukin1 beta (IL1-β) also to compare the route of vaccination by aerosol and ocular route for ND vaccine. The study design as follow : {G1: is positive control given EOs(6g) only; G2: given EOs(6g) with ND vaccine by aerosol route; G3: given EOs(6g) with ND vaccine by ocular route; G4: given ND vaccine by aerosol route only; G5: given ND vaccine by ocular route only; G6: is control negative not treated and not vaccinated}, Results of of IL-1β At 11 days old, levels of G5 were higher than levels at P(≤0.01). At 20 and 29 days old, also levels of G5 were higher than levels at (P≤0.01). Histopathological study revealed the following G1 Showed no clear histopathological lesion, G2 Trachea showed no clear pathological lesion while the lung showed very few area of congestion ,G3 Showed no clear pathological, G4 trachea showed mild odema formation, congestion and deciliation with few inflammatory cells infiltration, G5 Microscopically the trachea is normal while the lung show areas of congestion, G6 Was normal in architecture. In conclusion the EOs has anti-inflammatory effect and has protective effect after vaccination.

Key words: Fumite®Aroma , Newcastle disease , Interleukin 1 beta

INTRODUCTION

Newcastle disease virus has gained an interest of being used as anti-cancer virus in many researches concerning human (Obaid et al., 2021). It is endemic in iraq and causes respiratory and digestive lesion with 100% mortality(AL-Zuhariy, 2017; Al-Azawy et al., 2018). So vaccination alone is not enouth, therefore EOs can act as immunomodulatory and immunostimulatory at the same time (Shauqi and Omran, 2018) The process of Essential oil inhalation as vapor is a quick, practical, safe, easy and comfortable technique (Lee, 2016). Aromatherapy can be used as an immunomodulatory and immunostimulatory effect in some aromatic compounds (Ulaiwi, 2019).Oregano has been used for a long it is also a reliable source of useful plant-based medicines as phytotherapy, it is worldwide in distribution.(Arabaci et al., 2021). Bergamot is part of The Rutaceae family includes the aromatic plant bergamot *Citrus bergamia* The fruit is grown around the world in different continents, it has respiratory relief effect (Karaca et al., 2007). Lemongrass is related to *Cymbopogon* spp. are grown is for their essential oils. from the Poaceae family of grasses. Around 180 species of the genus lemongrass exist, it has an anti-inflammatory effect (shah et al., 2011). The goal of this study is to evaluate anti-inflammatory effect of evaporated EOs and to detect whether there is good or bad effect on respiratory tract of chicken.

MATERIAIS AND METHODS

Study design

A total of three hundreds (300) broiler chicks of the (Ross 308 strain) one day old, were adopted. They were purchased from a licensed hatchery and divided into six equal groups each group in a separated room. The experiment lasted for 35 days as follows:

G1: Fifty chick's(Control+) exposure to evaporation of EOs at the recommended dose at 9,18 and 27 days only
G2: Fifty chick's exposure to evaporation of EOs at 9,18 and 27 days before 1hr of (NDV)vaccination (spray method).
G3: Fifty chick's exposure to evaporation of essential oils at 9,18 and 27 days old before 1hr of (NDV)vaccination (eye drop method).
G4: Fifty chick's vaccination of (NDV) (spray method) at 9,18 and 27 days old only.
G5: Fifty chick's vaccination of (NDV) (eye drop method) at 9,18 and 27 days old only.
G6: Fifty chick's (control –ve) no evaporation and no vaccinations.

Experimental location
This experiment was carried out at chicken farm in Al-Fallujah university, College of the Veterinary Medicine the rooms was 2×3×3 for each separated group. It was exactly 35 days in the interval of(12 December up to 14 of January 2021). The dose was 6g for each group according to company recommendation before 1hr of vaccination

Vaccination
Live attenuated vaccine were given on (9, 18, 27 day old) (ND B1 BIO-VAC B1®,ND Clone 30 CLONE®, ND LaSota AVI ND LaSota® respectively.

Serum sampling
At the age of 3 day old 10 birds were sacrificed to measure the material immunity in order to decide the vaccination program, IL1β the serum samples taken at the age of 11,20 and 29 respectively(Gao et al., 2020). ELISA was to detect the parameters.

Technique of evaporation of essential oils
The process of evaporation was done on the basis of company instruction which is based on the reaction between a special igniter and the powder of essential oils to produce an evaporation that can be inhaled from chickens for about half an hour with regard to all fans and lights should be turn off, the doors should be closed.

Histopathological sampling
Samples with dimensions of 1cubic (cm) were collected from lung and trachea, 3 samples from each group at the end of experiment(day 35), Hematoxylin and Eosin (H & E) stain was used to stain all tissues, and the examination of tissues was done with the aid of light microscope for detection of histopathological changes (Suvarna et al., 2016).

Statistical analysis
The software Statistical Analysis System (SAS, 2012) was used to determine the effect of variables factors on the study parameters. Significant comparisons among means were performed using the Least Significant Difference -LSD test (Analysis of Variance-ANOVA).

RESULTS
Results of interleukin 1 beta
The current results of IL-1β revealed that at 11 days levels in G5 (299.854) were the highest followed by G4, G3 and G2 (259.319, 243.799 and 242.101 respectively) with statistically highly significance at the levels of P≤0.01 Table (1). There was no significance when comparing levels of G4, G3 and G2. Levels in G1 (112.904) and G6 (117.747) were the lowest (P≤0.01) when compared to other groups and there was no statistically significance between the results of these two groups. At 20 days old, levels in G5 (338.107) were the highest followed by G2, G3 and G4 (256.124, 266.868 and 263.314 respectively) and then followed by G6 (192.113) and then G1 (120.564) with highly significance at the levels of P≤0.01. There was no statistically significant difference when comparing levels of G2, G3 and G4. At 29 days old, levels in G5 (369.306) were the highest followed by G2, G3 and G4 (260.522, 271.961 and 266.060 respectively) and then followed by G6 (196.866) and then G1 (123.107) with highly significance at the levels of P≤0.01. There was no statistically significant difference when comparing levels of G2, G3 and G4.There was no significant difference when comparing the results of IL-1β in G1,G2 and G4 ,Results at G3 at 29 days old (271.961) was higher than levels at 11 days old (243.799) with significant difference (P≤0.05) results in G5 and G6 showed highly levels at 20 and 29 when compared to 11 days old with a significance at (P≤0.05).

Table (1) Results in different ages and groups of inerleukin 1 beta

	Mean ± SE of IL-1β (ng/L)			**LSD value**
Group	**11 Days Old**	**20 Days Old**	**29 Days Old**	
G1	112.904 C a	120.564 D a	123.107 D a	17.55 NS
G2	242.101 B a	256.124 B a	260.522 B a	22.74 NS
G3	243.799 B b	266.868 B ab	271.961 B a	26.37 *
G4	259.319 B a	263.314 B a	266.060 B a	26.49 NS
G5	299.854 A b	338.107 A a	369.306 A a	32.08 *
G6	117.747 C b	192.113 C a	196.866 C a	28.53 *
LSD value	36.02 **	45.93 **	41.62 **	---
Means with different capital letters in the same column and small letters in the same row are significantly different. * (P≤0.05), ** (P≤0.01).				

Results of histopatholgy

Histopathological findings of respiratory tract at 35 day old in both grossly and microscopically revealed that in G1 Showed no clear pathological lesion both grossly and microscopically (Figure.1), while G2 microscopically in trachea was normal, except the lung showed very few area of congestion microscopically when compared to vaccinated groups alone with ocular route i.e (G4) (Figure. 2), the G3 also showed no clear pathological lesion (Figure. 3), on other hands in vaccinated groups G4 trachea showed mild odema formation , congestion and deciliation with few inflammatory cells infiltration and the lung shows areas of congestion(Figure. 4), moreover G5 the lung contain an area of congestion while trachea is normal (Figure. 5). Finally G6 Was normal in architecture which is the control group (Figure.6).

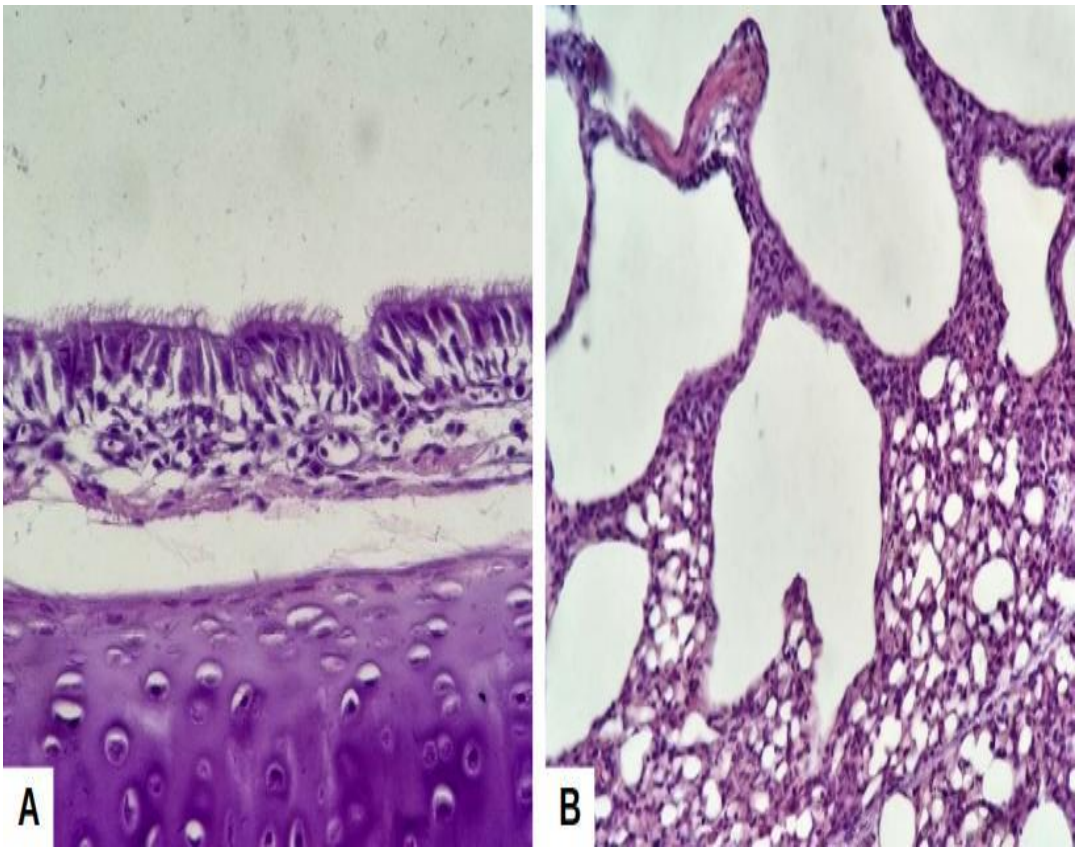

Figure .1 Photomicrograph of trachea from chicken in group 1 showed normal histological appearance in trachea(A) and lung(B). H&E X40

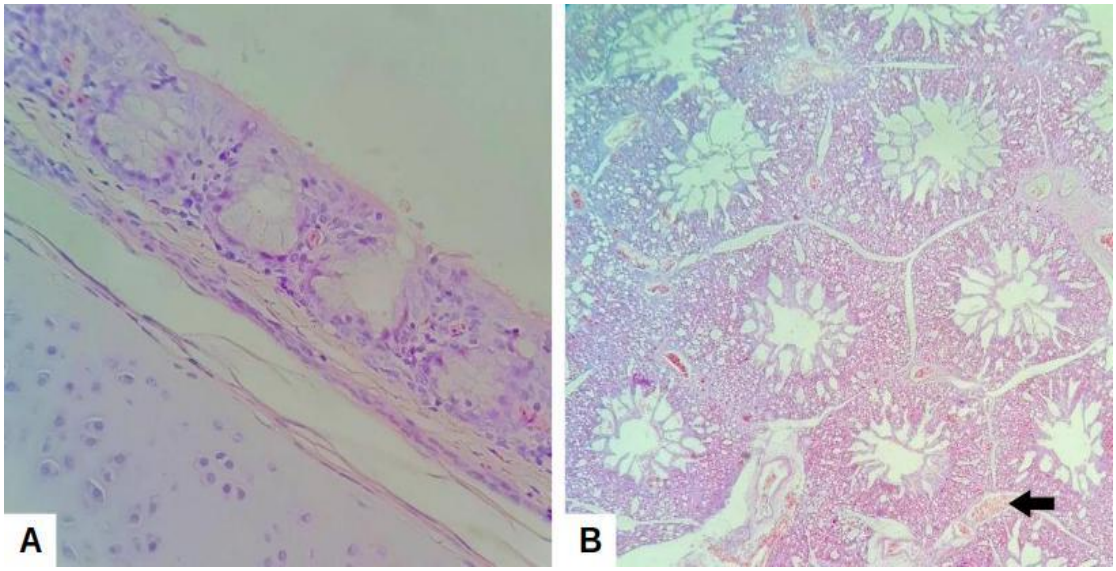

Figure .2 A-Photomicrograph of trachea from chicken in group 2 showed normal histological appearance . H&E X40
B- lung from chicken in group 2 showed few area of congestion(arrow). H&E X100

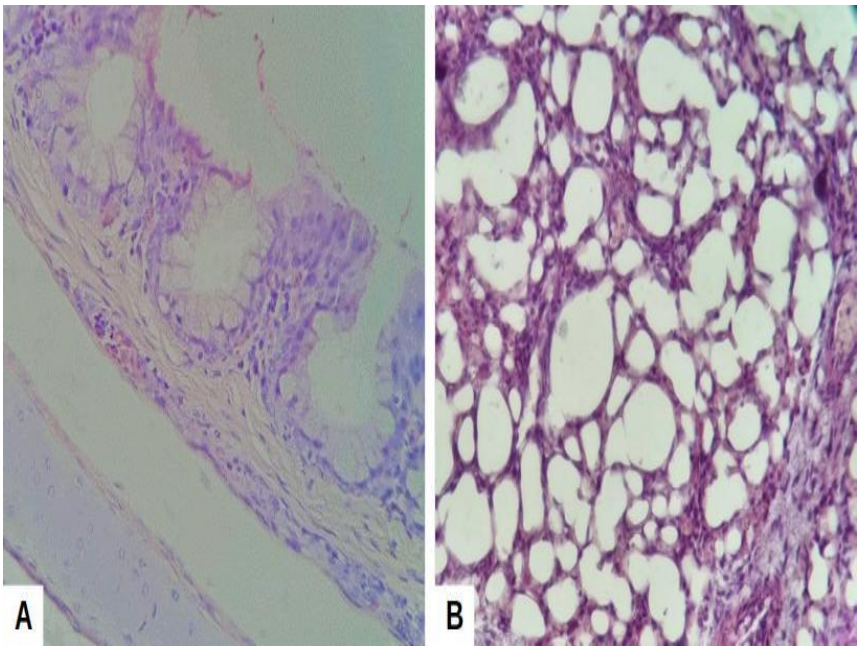

Figure .3 Photomicrograph of trachea from chicken in group 1 showed normal histological appearance in trachea(A) and lung(B). H&E X40

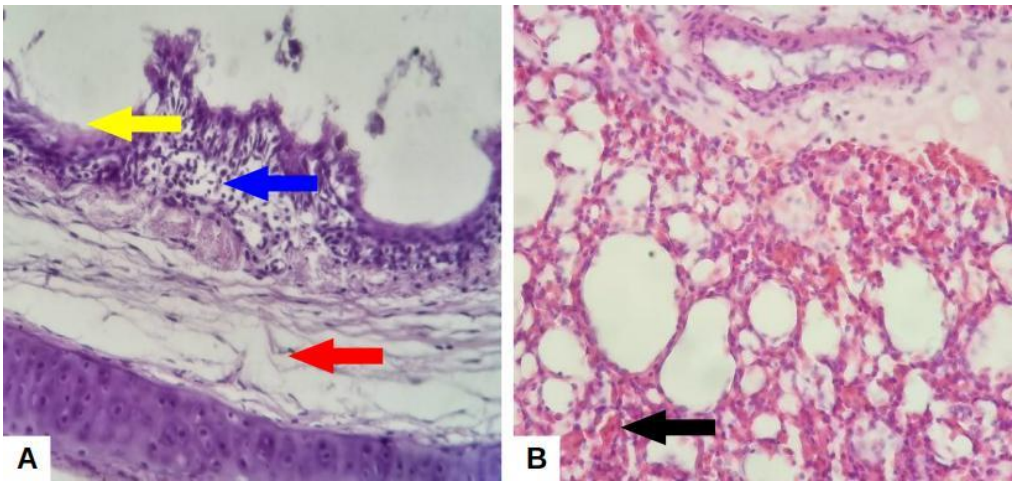

Figure .4 A- Photomicrograph of trachea from chicken in group 4 showed sub epithelial congestion with mild inflammation(Blue arrow), odema(Red arrow) and deciliation(Yellow arrow) . H&E X40. B- Photomicrograph of lung from chicken in group 4 shows area of congestion(Black arrow) . H&E X10

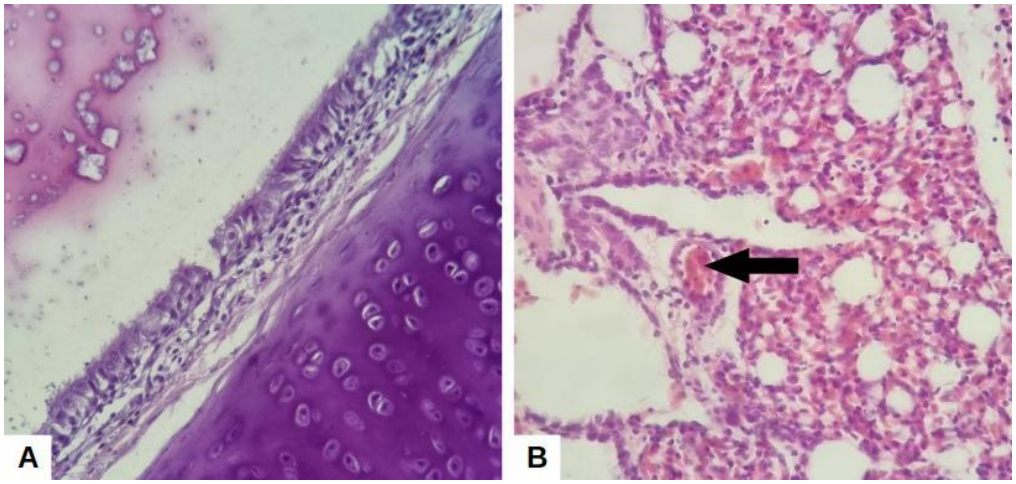

Figure. 5 A-Photomicrograph of trachea from chicken in group 5 shows no clear lesion. H&E X10. B- lung from chicken in group 5 an area of congestion in lung tissue(black arrow). H&E X40

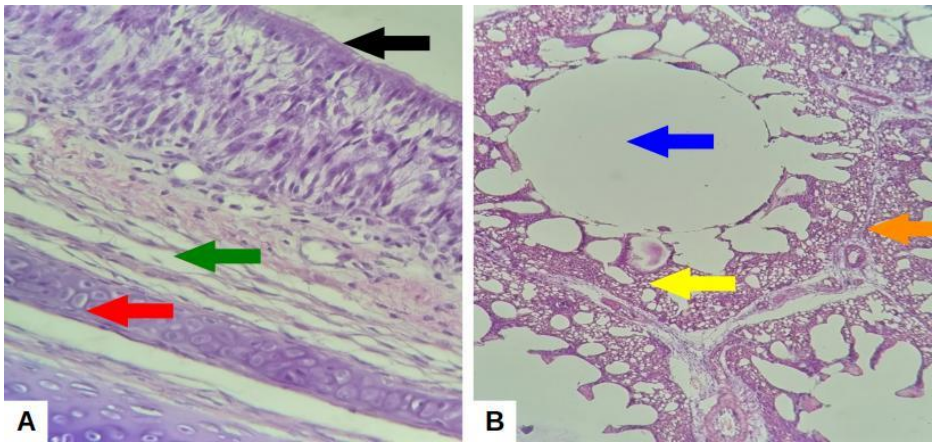

Figure .6 A-Photomicrograph of trachea from chicken in control showed normal histological appearance, ciliated, pseudostratified columnar epithelium (Black arrow) loose connective tissue of lamina proparia-submucosa (Green arrow) perichondrium(Red arrow) . H&E X40 B-normal histological appearance, parabronchial wall (Yellow arrow) interparabronchial septum (Orange arrow) parabronchus(Blue arrow). H&E AX10 BX40

DISCUSSION

The titer of IL1β that exposed to evaporated EOs was lower than non treated this could be attributed to the active ingredients that are present in this agree with Sforcin et al. (2009) who revealed that LEO can reduce IL-1β, IL-6 from macrophage additionally some EOs including ,the monoterpenes in LEO Additionally, it decrease the production of the cytokines IL-1β, IL-6 and TNFα and Cyclooxygenase-2. Moreover Feng et al. (2021) revealed that OEO act as an anti-inflammatory property had shown that TNF-α, IL-1β, and IL-6 are inhibited from macrophage of chicken (Zhang et al., 2022). Additionally some ingredients such as Limonene which is found in BEO can lessen IL-1β production (Chi et al., 2013). Moreover Cheng et al. (2018) proved that Cavacrol can reduce macrophage recruitment, alveoli size and the decrease pro-inflammatory cytokines of IL-1β, IL-6, IL-8, and IL-17, carvacrol and OEO lowered the inflammatory responsesi.e immunomodulatory. Recently Senthil Kumar et al. (2020). Proved that Geranium and lemonin shows activity against Angiotension enzyme 2 in epithelial cells thus may lower cytokines storm. But Mirghaed et al. (2020) claims that BEO or extract in the meals of several fish species might actually enhance the expression of IL-8 IL-1b and TNF-a. Vaccinated groups with eye drop route given Fumite® Aroma was lower than that with eye drop vaccine only which is related probably to an anti-inflammatory effect of BEO shown in this study confirms its use as an anti-inflammatory agent , the main components of the volatile fraction of BEO, anti-

inflammatory effect of BEO due to one or several compounds which act in synergistic action (Abdelli et al.,2018). Monotepenes like 1,8-cineole, α-terpineol, geraniol, citronellol, citral that are found in Fumite®Aroma which functions as a potent TNF-α and IL-1β inhibitor as a cytokine inhibitor to regulate airway mucus hypersecretion, recommending to lessen asthma severity, sinusitis and pneumonia (de Cassia da Silveira et al ., 2013).

While histopathological results showed that linalool which found in all of Fumite®Aroma product of EOs lowered the generation of TNF-α and IL-6 in bronchial alveolar lavage fluid, macrophages and heterophils, clearly show positive impact on Pulmonary tissues and edema of lung ,In conclusion, linalool can Blocking nuclear factor Kappa-light-chain-enhancer of activated B cell and Mitogen activated protein kinase (Huo et al., 2013). Alternatively bergapten and citropten from BEO have been shown by other studies to be potent inhibitors of IL-8 expression, they have been researched as potential therapy options to lessen lung inflammation in cystic fibrosis patients (Ferlazzo et al., 2016). Similarly LEO contain monoterpenes 1,8-cineole, α-terpineol, geraniol, citronellol, citral which act as anti-inflammatory which released in tissue damage for this reason the augmented EOs with vaccine was better than vaccinated birds only without EOs (Saied et al., 2021). Besides OEO may have protective effect to the chicken of of clinical signs probably due to its immunomodulatory effect on releasing of cytokines, it can protect against cytokines storm from TNF-α, IL-1β and IL-6 even if they are part of immune response, but presence in massive amount could lead to mucosal epithelial cell injury or necrosis (Maurya et al., 2021). Also of OEO can block angiotensin conversion which may act as antiviral agent (Carino et al., 2020). Alternatively OEO can act as decongestant to relieve respiratory symptoms when it is given to broiler chicken (Abu El Hammed et al., 2021). Indeed there could be respiratory lesion following application of live ND vaccination it is noticeable on trachea following 3 days of vaccine it can be attributed to vaccine reaction mostly after aerosol G4, ocular route maybe less reaction(G5) (Jarad et al., 2016; Ahmed and odisho, 2018). Therefore bergapten which is component of BEO attenuated pathological changes in other meaning lessen odema and congestion and alveolar septa thickening respiratory tract (Aidoo et al., 2019). Also Limonene which constitute BEO prevent respiratory injury by lower cytokines storm, lower secretion and allergic reaction (Chi et al., 2013). On other hand a study revealed carvacrol ,thymol and p-cymene role pulmonary emphysema which can show improvement of lung tissue in broilers when the management is bad (Games et al., 2016; Thajel and Ulaiwi, 2017). Regarding Linalool which

is found in all the product of this study reduced lung inflammation by inhibiting the inflammatory cells infiltration and the production of TNF-α, IL-6, IL-1β and IL-8, thus can reduce lung inflammation and injury as noted in groups that received the product of EOs (Ma et al., 2015). Additionally Linalool could also reduce nitric oxide synthase, which decrease nitric oxide which is responsible for signs of inflammation like odema and hyperemia and may protect the system also (Peana et al., 2008). Another study revealed that evaporated EOs can reduce air-borne microbes which in term reduce the propability of pneumonia or airsacculitis (Lahlou, 2004). Correspondingly the EOs for example LEO can be used to treat pneumonia and flu like symptoms (Acimovic et al., 2019). A study found that OEO ingredients which is carvacrol regulates cytokine production, inhibits reactive oxygen species, and stop eosinophils, monocyte and other cells attraction in respiratory system which in term protect respiratory system from allergic reaction (Gandhi et al., 2020). Furthermore after adding EOs to the feed, there was a reported increase in lysozyme activity which can protect if there is any secondary bacterial infection for example E.Coli which is endemic in Iraq (Zhang et al., 2020; Hameed et al., 2022). Recent investigations suggest that citral which is the major terpenoid in the EOS, has antioxidant effect as well (Anggraeni et al., 2018). Similarly the ingredients of OEO can provide anti-inflammatory processes that exhibit antioxidant also the oxidative stress is known to do damage to body tissues (Eler et al., 2019). Finally α-Terpinene, d-Limonene, p-Cymene, Linalool, Thymol are part of OEO which can prevent Reactive oxygen metabolite ,cytokines damage, proteolytic enzymes and inflammatory cells recruitment in lung which can lower pneumonia damage (wu et al., 2012).

Conclusion

It shows moderate maintenance of the tissues of respiratory tract during vaccination of ND that has been shown by histopathology and has anti-inflammatory effect by measuring IL-1β levels.

DECLARATION

Acknowledgments

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Authors' contribution

Waleed Hamed Farhan contributed to the final establishment of the experiment and the writing of this manuscript, Amjed Hussein Ulaiwi has contributed to data analysis and the design of the experiment as well as supervising during the experiment.

Competing interests

The authors have declared that there are no conflict of interests to the products that are listed in the manuscript.

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