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Animal Health and Well Being

Effects of turmeric, ginger, cinnamon, and garlic essential oils on HSP70, NFκB, oxidative DNA damage, inflammatory cytokines, and oxidative markers in broiler chickens

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Abstract

In recent years, the use of natural bioactive compounds derived from spices has garnered significant interest in poultry production due to their potential to modulate immune responses and oxidative stress. An investigation into the roles of spices essential oils (EO) on inflammatory cytokines, HSP70 and oxidative markers of broiler chickens was conducted in this study. Four spices consisting of garlic, ginger, turmeric, and cinnamon were processed to obtain their respective EO. Two hundred 1-d-old arbo acre broilers were allotted to 5 treatments consisting of B1 (control), B2 (garlic EO), B3 (ginger EO), B4 (Turmeric EO), and B5 (cinnamon EO), with EOs administered to drinking water at 30% (v/v) in a 49-d trial. Blood was sampled for assessment of hematological parameters, and serum obtained were assayed for inflammatory cytokines, antioxidant activities, nuclear factor kappa B (NFκB), 8-hydroxydeoxyguanosine (8-OHdG), and heat shock protein 70 (HSP70) levels using standard procedures. Results obtained revealed that cinnamon EO enhanced erythrocytic indices, leukocyte profile, catalase, glutathione peroxidase and interleukin 10, lowers interleukin 1 beta (IL-1β) and interferon gamma (IFN-γ), enhanced HSP70 and higher 8-OHdG levels in chicken. Garlic EO enhanced monocytes and superoxide dismutase, while reduced IFN-γ and HSP70, but increased IL-1β and tumor necrosis factor alpha (TNF-α) **NFκB** in broiler chickens. Ginger EO also enhanced erythrocytic indices, total antioxidant activity, lowered IFN-γ and lipid peroxidation, while turmeric EO enhanced total antioxidant activity, catalase and lowered IFN-γ and increased 8ohdg in broiler chickens. In conclusion, this study revealed that ginger and turmeric EO were more beneficial in preventing oxidative DNA damage, cinnamon EO enhanced serum oxidative status and lowered pro-inflammatory cytokines, while garlic EO reduced HSP70 in broiler chickens.

Lay Summary

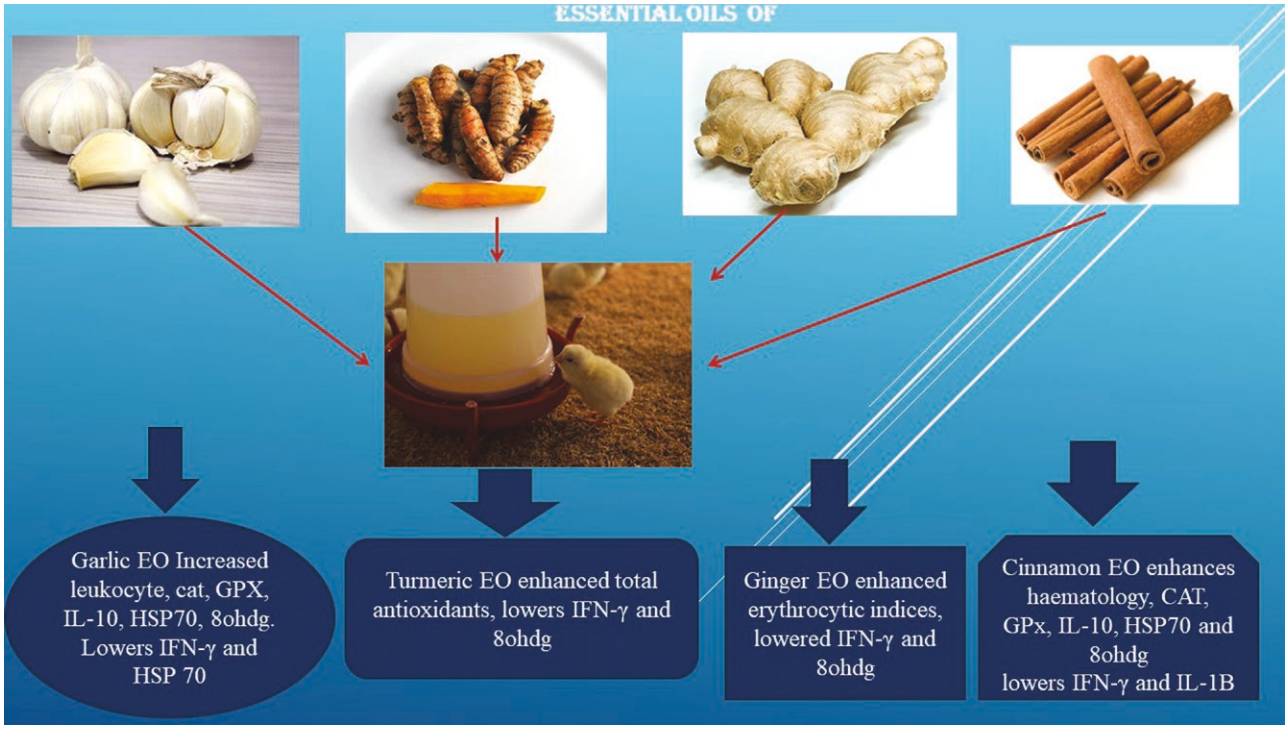
In recent years, scientists have been exploring natural compounds from spices for their potential health benefits in poultry. This study focused on the effects of essential oils (EO) from 4 spices—garlic, ginger, turmeric, and cinnamon—on the immune responses and stress levels of broiler chickens. Researchers conducted a 49-d trial with 200 broiler chickens, allocated into five groups. One group served as the control, while the other 4 received drinking water infused with EO from either garlic, ginger, turmeric, or cinnamon. The results showed that cinnamon EO improved several health indicators, including red and white blood cell counts, antioxidant enzyme levels, and anti-inflammatory markers, while reducing harmful inflammatory proteins and stress proteins. Garlic EO also had positive effects on monocytes and granulocyte counts and some antioxidants but increased certain inflammatory markers and stress proteins. Ginger EO improved packed cell volume and reduced both harmful inflammatory protein (INF-γ) and oxidative DNA damage. Turmeric EO boosted overall antioxidant activity and also reduced inflammatory proteins and DNA damage. Conclusively, ginger and turmeric EO were most effective in protecting against oxidative DNA damage. Cinnamon EO improved overall oxidative status and reduced inflammation, while garlic EO decreased stress proteins in broiler chickens.

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Graphical Abstract



Keywords: Pro-inflammatory, Antioxidant enzymes: Interleukins, Cinnamon, Turmeric

Abbreviations: 8-OHdG, 8-hydroxydeoxyguanosine; DNA, deoxyribonucleic acid; EO, essential oils; GPx, glutathione peroxidase; Hb, hemoglobin; HSP70, heat shock protein 70; IFN-γ, interferon gamma; IL-10, interleukin 10; IL-1β, interleukin 1 beta; MCH, mean cell hemoglobin; MCHC, mean cell hemoglobin concentration; MCV, mean cell volume; MDA, maloniadialdyde; NFκB, nuclear factor kappa B; PCV, packed cell volume; PUFA, poly unsaturated fatty acid; RBC, erythrocyte; ROS, reactive oxygen species; SOD, superoxide dismutase; TAC, total antioxidant capacity; TGF-β, transforming growth factor beta; TNF-α, tumor necrosis factor alpha; WBC, leukocytes

Introduction

The global push toward reducing or eliminating antimicrobial growth promoters in poultry production has fueled interest in alternative methods for maintaining bird health and performance. Historically, medicinal herbs and spices were utilized as natural additives due to their therapeutic properties (Frankic et al., 2009). These natural supplements, known for their low toxicity and absence of residual properties, have demonstrated potent antimicrobial and antioxidant activities. Consequently, they are increasingly recognized as valuable alternatives in animal production (Khan et al., 2012). Herbs and plant extracts has been characterized as alternatives of antibiotics, and have been utilized in livestock and poultry production (Daramola et al., 2020; Ayodele 2021; Jimoh et al., 2021, 2022; Oloruntola et al., 2022).

Traditional poultry feed ingredients, such as maize and soybean meal, are often deficient in antioxidants, which are crucial for mitigating oxidative stress and inflammation (Habibi et al., 2014). Insufficient antioxidant levels can lead to oxidative damage and inflammatory responses, influencing overall health and immunity (Huang and Lee, 2018). Aromatic compounds extracted from spices, known as essential oils (EOs), have shown promise in addressing these issues. EO possess various biological properties beneficial to poultry, including anti-inflammatory and antioxidant effects (Bento et al., 2013; Zhang et al., 2019). These oils can modulate immune responses and improve growth, immune status, and gut health in chickens (Adewole et al., 2021; Su et al., 2021). Phytogenic supplements are capable of modulating humoral responses in poultry and their bioactive components are reported to control NFκB and/or MAPKs pathways (Liang et al., 2014). EO have beneficial effects on chicken immunity by promoting immunoglobulins, enhancement of lymphocyte, as well as the enhancement of interferon-γ secretion (Adaszyńska-Skwirzyńska and Szczerbińska, 2017). Aromatic oils commonly utilized in rearing chicken are oils from *Curcuma longa*, *Cinnamomum zeylanicum*, *Zingiber officinale*, *Allium sativum*, and *Syzygium aromaticum* (Adaszyńska-Skwirzyńska and Szczerbińska, 2017).

Cinnamaldehyde of cinnamon EO has proven to be a potent antimicrobial (Chang *et al*. 2001). Cinnamon EO in Japanese quails' diet alleviates the negative consequences of thermal stress and activates antioxidant mechanism in the protection of the organs (Simsek et al. 2013). Cinnamon EO lower lipid peroxidation and its effects in the animals (Keshvari et al., 2013). The active ingredients in turmeric possess antioxidant, antibacterial and anti-inflammatory activities, and has also demonstrated that the antibiotic activities that impede the proliferation of *C. perfringens* (Ali et al., 2020). Inclusion of garlic and ginger in chicken diets are known to positively influence immunity and aid digestion in poultry (Gardzielewska et al., 2003). Active ingredients in ginger EO possess antioxidant and antimicrobial activity that has been documented to be potent in poultry production (Habibi, et al., 2014). Ginger and garlic EOs are easily accessible, cheap, safe, effective and are additives capable of promoting anti-oxidant, anti-microbial, anti-inflammatory, hepato-protective, and hypolipidemic effects in birds (Oluwafemi et al., 2021a).

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There are reported cases, which shows that EOs when utilized as growth promoter in broiler production, fail to improve performance, and worsen growth in some cases (Zeng et al., 2016). Hence, there is the need to appropriately select, compose appropriate dosage of EO for supplementation (Su et al., 2021). A combination of EOs has been evaluated in vitro, and displayed more intense antibacterial activity than single EO (Bento et al., 2013). However, before suggesting synergistic effects between single activities, more in vivo trials are needed to unmask the mechanisms of action of individual EOs in farm animals. Despite numerous studies on the addition of EOs in diet, there exists a paucity of information on the effects of oral administration of these substances (Liu et al. 2019). Research is required to clarify the mechanisms by which these EO influence poultry health and performance, addressing the need for more in vivo trials to better understand their efficacy and potential synergies.

Thus, this study investigates the comparative effect of oral administration of EOs of turmeric, ginger, cinnamon and garlic on hematology, inflammatory cytokines, oxidative status, and cellular stress markers of chickens.

Materials and Methods

Ethical approval

The conditions and guidelines for the use of animals and animal operations for the purposes of experiments were accepted (AP/REC/2021/004) by the research and ethics committee of The Federal Polytechnic's Animal Production Department in Ado Ekiti town, Ekiti State, Nigeria.

Phytogenics processing and Evaluation

Fresh and mature garlic, turmeric, ginger, and cinnamon were procured from a spices market in Ado-Ekiti. They were peeled, diced and homogenized in water (40%, w/v) and filled into a boiling chamber of a Clevenger-type apparatus for hydrodistillation. The oil extracted was later dehydrated and preserved in airtight vials below 4°C.

Experimental animals and management

Two hundred (200)-d-old arbo acre broilers were obtained from an illustrious hatchery. The birds were allotted by weight to five treatments of 10 replicates and four birds/replicate in a completely randomized design and allotted to B1: no supplement, B2: garlicEO, B3: ginger EO, B4: turmeric EO, B5: cinnamon EO.

An experimental basal diet was formulated to fulfill the nutrient requirement of the birds at the 2 production phases as shown in Table 1. The essential oils of the spices were supplemented (30 ml/100 ml) into drinking water for the birds throughout the study. Birds were fed ad libitum and fresh water was offered daily in a feed trial that lasted for 49 d. The hatchery recommended program of vaccination were followed and medication was administered throughout the study.

Blood sample collection

On the 49th day of the trial, blood samples were collected from 3 birds/replicate into plain and EDTA bottles for obtaining serum and plasma, respectively. Hematological indices (packed cell volume (PCV), erythrocyte (RBC), hemoglobin (Hb), mean cell volume (MCV), mean cell hemoglobin (MCH), mean cell hemoglobin concentration (MCHC), leukocytes (WBC), granulocytes, lymphocytes, and monocytes) were assessed in each blood sample as outlined by Ewuola et al. (2012). Blood samples in plain tubes were centrifuged and serum obtained using standard procedures and stored at −20 °C until analysis.

Serum were assayed for total antioxidant capacity (TAC), glutathione peroxidase (GPx), lipid peroxidation, catalase, and superoxide dismutase (SOD) as outlined by Jimoh et al. (2019).

Table 1. Gross composition of broiler starter ration

Ingredient	Starter diet	Finisher diet
Maize	58.5	66.5
Groundnut cake	9.8	8.3
Soyabean meal	25	19.5
Fishmeal	3.1	2
Methionine	0.25	0.25
Lysine	0.25	0.25
Oyster shell	1.5	1.5
Limestone	1.1	1.2
Premix	0.25	0.25
Salt	0.25	0.25
Total	100	100
Dry matter, %	85.848	85.546
Crude protein, %	22.992	18.575
Metabolizable energy, kcal/kg	3031.27	3086.43
Ether extract, %	3.9425	3.9305
Crude fiber, %	3.316	3.0325
Lysine, %	1.37755	1.17005
Methionine, %	0.60564	0.56059
Calcium, %	1.17455	1.15925
Phosphorus ,%	0.31525	0.47845

Inflammatory cytokines, cellular stress markers, and Oxidative DNA damage

Assays were performed on serum samples using enzyme-linked immunosorbent assay (ELISA) with proprietary kits. The procedure for each assay was followed as outlined in the respective kit instructions..

- Interferon gamma (IFN-γ) ELISA Kit, Catalog No.: E-EL-R0009 (Elabscience Biotechnology Inc., USA).
- Tumor necrosis factor alpha (TNF-α) ELISA Kit, Catalog No.: E-EL-R0019 (Elabscience Biotechnology Inc., USA).
- Interleukin 1 beta (IL-1β) ELISA Kit, Catalog No.: E-EL-R0012 (Elabscience Biotechnology Inc., USA).
- Interleukin 10 (IL-10) ELISA Kit, Catalog No.: E-EL-R0015 (Elabscience Biotechnology Inc., USA).
- Heat shock protein 70 ELISA Kit, Cat. No. E0522Ra (Bioassay Technology Laboratory, www.bt-laboratory.com, Yangpu District Shanghai, China).
- Nuclear factor kappa B (NFκB) Elabscience E-EL-R0674. Elabscience Biotechnology Inc.
- 8-Hydroxydeoxyguanosine (8-OHdG) Elabscience E-EL-0028. Elabscience Biotechnology Inc.

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Statistical analysis

The statistical model applied was as follows: $Y_{ijl} = \mu + B_i + e_{ijl}$

where Y_{ijl} represents the value of haematological indices, serum oxidative status, inflammatory cytokines and cellular stress markers measured in the *l*th animal; μ is the overall mean for each character; B_i is the fixed effect of *i*th spices essential oils administered; and e_{ijl} is the random residual effect.

Data obtained from the study were subjected to generalized linear model procedure of one-way analysis of variance using to statistical analysis software IBM SPSS 20.

Results

The results for the hematological indices of broiler chickens administered with essential oils of spices are shown in Table 2. The PCV of birds on B2 and B1 were statistically ($P > 0.05$) similar and significantly ($P < 0.05$) lower than that of birds on other treatment. Hemoglobin of birds of B5 were significantly ($P < 0.05$) higher than those of birds in other treatments; B1, B2, B3, and B4, which share statistically ($P > 0.05$) similar values. MCV and MCH of birds on B4 and B1 were statistically ($P < 0.05$) lower than other treatments, while those of birds on B2, B3, and B5 share statistically ($P > 0.05$) similar values. MCHC of birds on B5 was significantly ($P < 0.05$) higher than values of birds on B1 and B2. Leukocytes of birds on B5 was significantly ($P < 0.05$) higher than Birds on B4. Granulocytes in birds on B5, B2 and B1 were statistically ($P > 0.05$) similar and significantly ($P < 0.05$) higher than those of birds on B3 and B4. The lymphocytes of birds on B5 were significantly ($P < 0.05$) higher than birds on B1 and B4. The monocyte of birds on B5 and B2 are statistically ($P < 0.05$) higher than those of birds on other treatment which share statistically similar ($P > 0.05$) values.

Serum oxidative status of broiler chickens administered with spices essential oils is shown in Table 3. Total antioxidant activity of birds on essential oil-based treatments were significantly ($P < 0.05$) higher than the control and B4 birds had significantly ($P < 0.05$) highest value. Lipid peroxidation was significantly ($P < 0.05$) higher in B1 birds than essential oil-based treatments, with B2, B3, B4, and B5 showing statistically ($P > 0.05$) similar values. Birds on B2 had significantly ($P < 0.05$) higher superoxide dismutase activity than birds on B1. Birds on B5 had significantly ($P < 0.05$) higher glutathione peroxidase and catalase activities than birds on other birds.

Inflammatory cytokines of birds on different spices essential oils are shown in Table 4. Interferon gamma of birds on B1 was significantly ($P < 0.05$) higher than those of birds on essential oil-based treatments. Interferon gamma of birds on spices essential oils were not significantly ($P > 0.05$) different from one another. Interleukin 1 beta and Tumor necrosis factor alpha of birds on B2 were significantly ($P < 0.05$) higher than those of birds on B1, B3, and B4, which share statistically ($P > 0.05$) similar values. Interleukin 10 of birds on B5 was significantly ($P < 0.05$) higher than birds on B1, B3, and B4, while B2 had the statistically ($P > 0.05$) least value.

Cellular stress markers of birds administered with spices essential oils are shown in Figure 1. HSP70 of birds on B5 was significantly ($P < 0.05$) higher, while birds on B3, B4, and B1 had statistically ($P > 0.05$) similar values, and the lowest values were obtained in birds on B2. NFκB of birds on B2 was significantly ($P < 0.05$) higher than those of birds on other treatments, with birds on B1, B3, B4, and B5 share

Table 2. Hematological indices of broilers administered spices essential oils

	B1	B2	B3	B4	B5	SEM
Packed cell volume, %	29.67^{b}	30.33^{b}	32.33^{a}	32.00^{a}	34.00^{a}	0.66
Erythrocyte (×10^{6}/L)	2.73	2.30	2.40	3.00	2.97	0.16
Hemoglobin, Hbg/dl	9.89^{b}	10.11^{b}	10.78^{b}	10.67^{b}	11.33^{a}	0.22
Mean cell volume, fL	108.58^{b}	135.88^{a}	137.53^{a}	110.19^{b}	123.28^{a}	6.26
Mean cell hemoglobin, pg/cell	36.19^{b}	45.29^{a}	45.84^{a}	36.73^{b}	41.09^{ab}	2.09
Mean cell hemoglobin concentration, g/dl	33.03^{b}	33.03^{b}	33.67^{ab}	33.33^{ab}	34.06^{a}	0.15
Leukocytes, ×10^{9}/L	2.30^{ab}	2.70^{ab}	2.23^{ab}	1.97^{b}	3.33^{a}	0.19
Granulocytes, ×10^{9}/L	0.93^{a}	1.01^{a}	0.72^{b}	0.60^{b}	1.05^{a}	0.09
Lymphocytes, ×10^{9}/L	1.34^{b}	1.63^{ab}	1.58^{ab}	1.33^{b}	2.21^{a}	0.11
Monocytes, ×10^{9}/L	0.03^{b}	0.06^{a}	0.04^{b}	0.04^{b}	0.07^{a}	0.01

B1: no supplement; B2: garlic essential oil; B3: ginger essential oil; B4: turmeric essential oil; B5: cinnamon essential oil.
^{a,b}Means with different superscripts are significantly different.

Table 3. Serum oxidative status of broilers administered spices essential oils

	B1	B2	B3	B4	B5	SEM
Total antioxidant activity, mmol/L	0.49^{c}	0.91^{b}	1.06^{b}	2.55^{a}	1.39^{b}	0.36
Lipid peroxidation, ×10^{-8} MDA/mg protein	0.55^{a}	0.27^{b}	0.20^{b}	0.34^{b}	0.20^{b}	0.08
Superoxide dismutase, U/min/mg protein	1.20^{b}	6.40^{a}	3.60^{ab}	3.60^{ab}	3.20^{ab}	0.63
Glutathione peroxidase, μg GSH/min/mg protein	14.16^{b}	14.44^{b}	14.30^{b}	13.81^{b}	17.58^{a}	0.90
Catalase, nm H_2O_2/min/mg protein	0.07^{c}	0.28^{b}	0.17^{bc}	0.25^{b}	0.36^{a}	0.05

B1: no supplement; B2: garlic essential oil; B3: ginger essential oil; B4: turmeric essential oil; B5: cinnamon essential oil.
^{a,b,c}Means with different superscripts are significantly different.

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Table 4. Inflammatory cytokines of broiler administered spices essential oil

	B1	B2	B3	B4	B5	SEM
Interferon gamma, ng/ml	43.09[a]	25.91[b]	29.85[b]	25.93[b]	23.45[b]	2.37
Interleukin 1 beta, pg/ml	55.85[b]	77.31[a]	58.54[b]	54.32[b]	40.58[c]	3.98
Tumor necrosis factor alpha, pg/ml	52.17[b]	72.18[a]	54.61[b]	50.73[b]	37.90[b]	3.72
Interleukin 10, pg/ml	59.65[b]	40.09[c]	55.58[b]	56.85[b]	78.15[a]	4.22

B1: no supplement; B2: garlic essential oil; B3: ginger essential oil; B4: turmeric essential oil; B5: cinnamon essential oil.
[a,b,c]Means with different superscripts are significantly different.

statistically ($P > 0.05$) similar values. 8oHdg of birds on B4 and B5 was significantly ($P < 0.05$) higher than birds on B1, B2, and B3, which share statistically similar values.

Discussion

The trends of results obtained in this study indicates that birds on ginger EO and cinnamon EO had higher erythrocytic indices. EO appear to maximize absorption of Fe and Cu ions and similar minerals resulting in enhanced bioavailability in the blood, which can enhance hemoglobin as reported by Ranwa et al. (2022) in quails. Lower MCH in turmeric EO groups obtained in this study is similar to that observed by Ranwa et al. (2022) that attributed decrease in MCH in Japanese quails on turmeric essential oil to increased hemoglobin and normal RBC size. Similarly, Al-Kassie (2009) reported that cinnamon oil improved RBC counts, PCV, and hemoglobin in broilers. The increase in hemoglobin and MCH values reflect the oxygen-carrying capacity of birds (Samantaray and Nayak 2022). Conversely, Abo Ghanima et al. (2020) and Saied et al. (2022) had reported that feeding diet containing cinnamon essential oils to chicken had no effect on RBCs, WBCs, Hb, and PCV. These could be attributed to the stimulating effects of the herbs on hematopoietic tissue or antibody production (Abd El-Latif et al., 2013) The inclusion of garlic EO did not influence the erythrocytic indices, which is in tandem with claims that the inclusion of garlic EOs to broilers diets have no effect on RBC (Abd El-Latif et al., 2013). Contrariwise, an increase in RBC of birds on garlic extract inclusive diets (Elnagar et al., 2003). But the claims that administration of garlic and ginger increases hemoglobin content when added at 10 ml/L water thrice weekly (Rehman et al., 2011) may indicate a possible synergistic effect of a combination of garlic and ginger. This is because the antioxidant properties of essential oils have been linked to an increased erythrocyte count in Japanese quails (Ranwa et al., 2022). The report of the present study agrees with the findings that the inclusion of turmeric EOs had no effect on MCHC levels in Japanese quails (Ranwa et al., 2022). In contrast, the findings of Oluwafemi et al. (2021b), who reported an improved erythrocytic profile in broilers with turmeric oil supplementation, do not align with the results of this study.

Birds on cinnamon EO had higher leukocyte profile in this study, which indicates the active components in stimulating synthesis and increased production of white blood cells and its differentials. This corroborates the works of Al-Kassie (2009), which showed that birds fed on diets containing cinnamon essential oils had higher WBC. Similarly, Sura (2018) reported that cinnamon resulted in an increment in the total counts of WBC of chicken. While, cinnamon (5.0 g/L) in drinking water was reported to positively modulate immune responses in 21-d-old broilers (Sadeghi et al., 2012). This agrees with reports that affirmed that supplementation with Cinnamon essential oils improved the immune function and antioxidative ability (Yang et al., 2019).

Reports that total and differential leukocytic count increase in response to garlic essential oils fed to broilers and laying hens (Ademola et al., 2011), probably due to the significant lymphocytosis (Abd El-Latif et al., 2013), are in agreement with this study. This can be attributed to the stimulating effects of the EOs on immunity and triggers immuno-competence in the chicken (Abd El-Latif et al., 2013). Contrariwise, Ranwa, et al. (2022) reported that turmeric essential oils influenced heterophils and heterophil: lymphocytes of quails.

Birds on essential oils had higher total antioxidant activity, antioxidant enzyme activities and lower lipid peroxidation rates compared to birds on control. The findings of this study indicates that the active compounds in the essential oil have antioxidant potency to mop-up free radicals and scavenge oxygen species. The reduction in lipid peroxidation could be a result of the enhanced activity of liver antioxidant enzymes (Yang et al., 2019). Likewise, ginger essential oil is rich in phenols, confers protection of cells against deleterious effects of Reactive oxygen species (ROS), leading to lower maloniadialdyde (MDA) level in quails (Herve et al., 2019). This is corroborated by claims that cinnamon oil supplementation in broilers led to decreased blood MDA levels, by the enhancement of antioxidant activities (Ciftci et al., 2010; Symeon et al. 2014). Moreover, Keshvari et al. (2013) claimed that cinnamon essential oils inhibit MDA production and are suitable to reduce lipid peroxidation and its effects on animals. Thus, cinnamon EOs markedly minimize MDA formation and are ideal candidates to inhibit lipid peroxidation and scavenge-free radicals in the body (Yang et al., 2019).

Birds on turmeric EO had the highest total antioxidant activity. Garlic EO enhanced SOD activity, cinnamon EO enhanced GPx and catalase activities better than other essential oils treatment groups. The differential mechanism of action of EOs on antioxidant enzymes could explain the total antioxidant enhancement and reduced lipid peroxidation in birds administered EOs. Our result is corroborated by reports that showed garlic essential oils to have a potency to increase SOD and GPx levels, which resulted in decreased MDA in chickens (Elbaz et al. 2022). Similarly, Chowdhury et al. (2018) reported that supplementation with cinnamon bark oil increased superoxide dismutase activities of broilers, while increased glutathione peroxidase and reduced malondialdehyde were reported as effects of cinnamon oil consumption by broiler chickens (Cifici et al., 2010). However, during exposure of chicken to high temperature, consumption of turmeric oil resulted in the increase of glutathione peroxidase activity (Akbarian et al., 2014). Other authors have also

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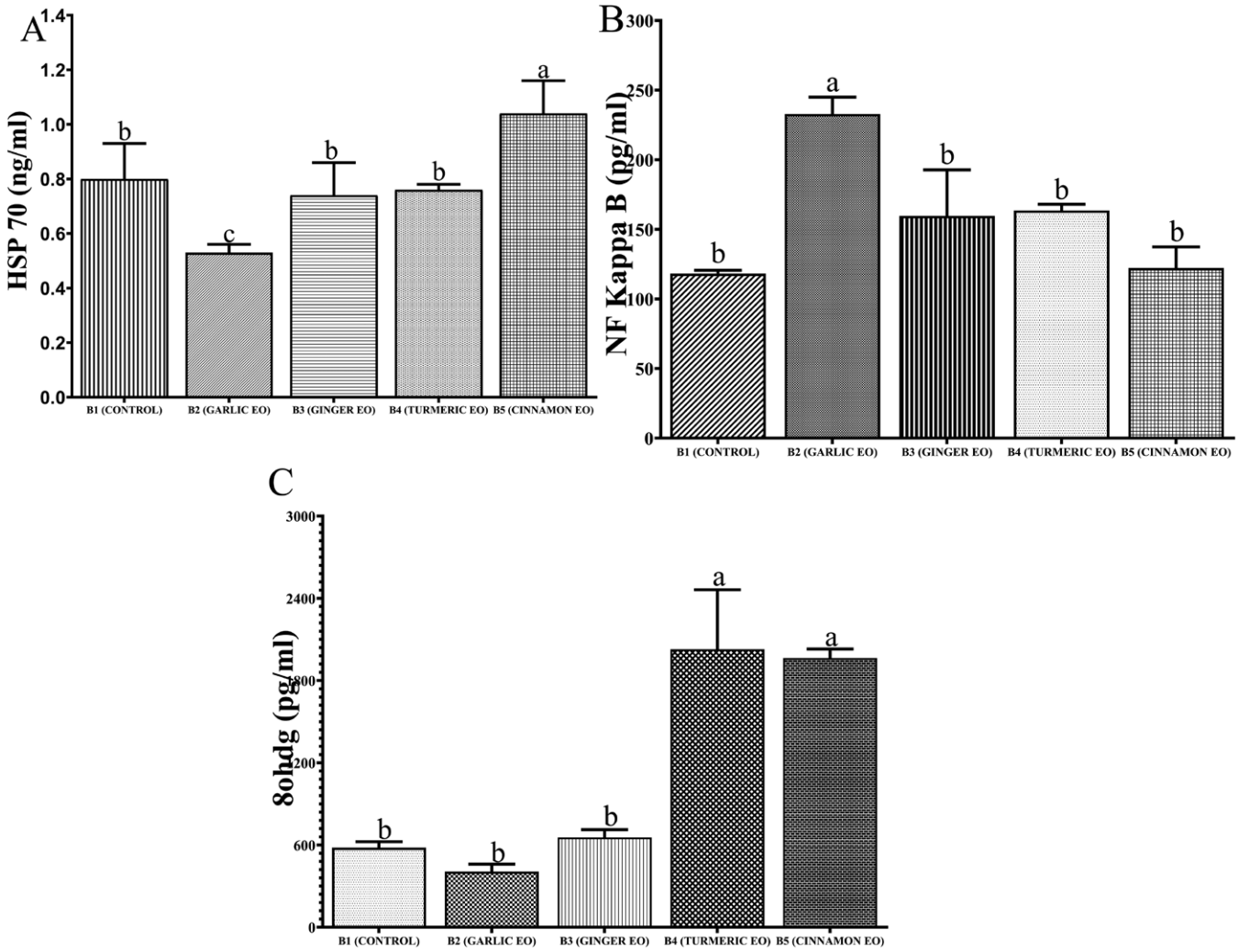


Figure 1. Cellular stress markers and oxidative DNA damage of broiler administered spices essential oil. The effects of treatment are shown in (A) heat shock protein 70, (B) nuclear factor kappa B, (C) 8-hydroxydeoxyguanosine. abc: means with different superscripts are significantly ($P < 0.05$) different; broiler chickens were administered spices essential oils in B1: no supplement; B2: garlic essential oil; B3: ginger essential oil; B4: turmeric essential oil; B5: cinnamon essential oil.

reported similar responses in lavender essential oil resulted in decreased lipid peroxidation resulting from increased superoxide dismutase and glutathione peroxidase (Yarmohammadi et al., 2020).

Although Tekeli et al. (2006) showed that ginger EO enhanced SOD and GPx but reduced MDA in different animal models, the result of this study indicates that the total antioxidant activity of birds administered with ginger essential oils was better than birds on control, while antioxidant enzymes were not influenced by ginger EO in comparison with those on control. The difference could be due to the dosage used in this study, which may have elicited different responses in other essential oils. However, claims by Habibi et al. (2014) that ginger EO increased TAC and decreased the MDA in chickens, corroborates the result of this study. The antioxidant system successfully adapts through upregulation of glutathione synthesis by phenolic compounds (Akbarian et al., 2014). Glutathione are usually present intracellularly at high concentrations and protect against the deleterious effects of lipid peroxidation (Nordberg and Arner, 2001).

GPx activity is more important in regulating low cellular hydrogen peroxide due to its much lower K*m* than catalase (Faix et al., 2009). It explains the mechanism of cinnamon EO inducing the highest GPx activity and accounts for the highest total antioxidant activity in birds in comparison with other EOs. Again, Faix et al. (2009) reported that *Cinnamomum zeylanicum* essential oil administration to chicken was effective to enhance only GPx activity and not SOD and catalase, which is in line with our result.

The high antioxidative profile and low MDA in birds fed on cinnamon EO supplements is indicative of their capability to remove free radicals, as well as their potency as a good natural antioxidant. Also, total antioxidant activity, SOD, GPx, and glutathione have been reported to be enhanced in growing quails fed on diets containing cinnamon essential oil and ginger essential oil (Ahmed et al., 2019). The trend of the result obtained in this study support claims that spices EO when used as antioxidant supplements eliminate free radicals, improves antioxidant profile, and inhibits lipid peroxide accumulation thereby resulting in higher cell membrane

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stability, reduce RBC hemolysis and beneficial contributions to hematopoiesis (Ranwa, et al., 2022).

Inflammation is responsible for regulating threats, reducing damage, containment, and healing. Afterward, the primarily secretion of pro-inflammatory cytokines such as IFN-γ and TNF is initiated as the first line of defense in the immune system (Kaiser et al., 2006). Pro-inflammatory cytokines are mobilized during immune response resulting in inflammation and thereafter metabolic anomalies that result in tissue injury (Zhang et al., 2019)

In this study, IFN-γ activity was lower in birds on essential oils, suggesting the role of the spices essential oils to reduce pro-inflammatory cytokines in the birds. IFN-γ is the intermediary in innate immunity and controlling numerous pro-inflammatory factors like TNF-α (Muhl and Pfeilschifter, 2003). TNF-α is an essential intermediate agent between local and systemic inflammation. However, the administration of garlic to broiler chickens enhanced IL-1β and TNF-α and lowered IL-10. This is in line with Hanieh et al. (2010) that demonstrated that garlic enhances interferon, interleukins, and TNF-α. This is an indication of the immunomodulatory properties of garlic as reported by Elbaz et al. (2022) that the addition of garlic essential oils in chicken ration acts by immunomodulation of chickens via provision of nutrients required to synthesize antibodies in the development of immune system. Similarly, Hafez et al. (2022) observed that curcumin administration to chicken reduced pro-inflammatory cytokines (TNF-α, IL-2, IL-6) and improved antioxidant activity and immunity status. The result of higher IL-1β and TNF-α in the garlic EO group could be associated to reports that cinnamaldehyde enhanced mRNA expression of IL-1β, IL-6, IL-15, and IFN-γ (Huang and Lee, 2018).

EO rich in terpenoids and flavonoids are known to suppress the metabolism of inflammatory prostaglandins in animals due to strong anti-inflammatory properties (Krishan and Narang, 2014). IL-10 and transforming growth factor-β (TGF-β) are key anti-inflammatory cytokines that mitigate the inflammatory process by inhibiting the pro-inflammatory cytokines production (Kabploy et al., 2016).

Moreover, the result of this study on birds administered with cinnamon EO which had enhanced IL-10 is in agreement with reports that affirm that cinnamon oil and cinnamaldehyde prevents mRNA expression of IL-1β, IL-6, and TNF-α and increased IL-10 production (Huang and Lee, 2018). Su et al. (2021) also reported that EO addition increases anti-inflammatory cytokines (immunoglobulin) and enhanced antioxidant activity. A similar trend has been reported in other essential oils such as eucalyptus and peppermint EOs in water consumed by birds that enhanced cell-mediated and humoral immune response (Awaad et al., 2010).

Birds not administered with EO in this study had higher HSP70 than birds on garlic EO. This corroborates claims that chickens fed on a diet without EOs had elevated HSP70 (Akbarian et al., 2014). However, it is not similar for all EO, because birds on cinnamon EO had higher HSP70 than birds without EO. Similarly, dietary EO supplementation effects on the antioxidant activity and HSP70 expression in different tissues are in a complex pattern, thus indicating that EO effects depend on the age of birds, tissue, and EO (Akbarian et al., 2014). HSP70 of birds on cinnamon EO was higher than others, while birds on garlic EO had the lowest values. This study also observed a high association between lipid oxidation and HSP70 as reported by Mahmoud et al. (2004), indicating that key factors that induce HSP synthesis are associated with generation of ROS.

Pro-inflammatory cytokines, ROS, and mitogens produced by different stressors induce the activation of NFκB signaling (Zhao et al., 2013), tailored to the mobilization of other inflammatory cytokines and chemokines (Huang and Lee, 2018). NFκB of birds on garlic EO has the highest value across the groups. This is similar to the findings by Roth-Walter et al. (2014) that cinnamaldehyde elevated NFκB activation in LPS stimulated human cells. Usually, dietary antioxidants enhance the elimination of ROS and results in preventing the activation of the NFκB-mediated inflammation process (Jang et al. 2014). This could be attributed to the fact that the transcription factors AP-1 and NFκB involved in the stimulation of antioxidant enzymes and heat shock protein and its transcription factor are sensitive to the redox status of the cell (Khassaf et al., 2003; Akbarian et al., 2014). Hence, oral antioxidants intake may interact with antioxidant enzymes to maintain the state of cells and interfering with the activation of the transcription factors (Akbarian et al., 2014). Contrariwise, Youn et al (2008) reported that in vitro cinnamaldehyde suppress LPS-induced activation of NFκB. Similar to this study, reduced expression of TNF-α and NFκB by cinnamon in broiler chickens indicates anti-inflammatory activities of cinnamon in pathological conditions (Tabatabaei et al., 2015).

ROS toxicity could affect component of the DNA and against PUFA in the cell membrane to induce lipid peroxidation (Buonocore et al., 2010), leading to 8-hydroxy-2′-deoxyguanosine formation. Hence, 8-hydroxy-2′-deoxyguanosine is pivotal indicator of oxidative stress-induced DNA damage.

The reduction in 8ohdg of birds due to garlic and ginger essential oils as obtained in this study is an indication that the two EOs may be key in mitigating DNA damage (Beyzi et al., 2020). Resulting from the investigation of this study, it was observed that the administration of cinnamon EO enhances erythrocytic indices, leukocyte profile, catalase, GPx and IL-10, lowers IFN-γ and IL-1B, and enhanced HSP70, which could have led to higher 8ohdg in chicken. Administration of garlic EO tended to enhance leukocyte indices and SOD, which tends to lower IFN-γ, HSP70, but increased IL-1B and TNF-α, which could have led to higher NFκB and 8ohdg in broiler chicken. Also, the administration of ginger EO enhanced erythrocytic indices, and lowered IFN-γ and 8ohdg, while turmeric EO administration enhanced TAC, and lowers IFN-γ and 8ohdg in broiler chicken.

Conclusion

This study reveals that the administration of essential oils via drinking water to broiler chicken influenced inflammatory cytokines, and modulated serum, and DNA oxidative markers. The inclusion of ginger and turmeric EO was more beneficial in preventing oxidative DNA damage, cinnamon EO exerted positive effects on hematology, serum oxidative status and lowers pro-inflammatory cytokines while garlic EO enhanced leucocytic indices and reduced heat shock protein in broiler chicken.

Author Contributions

Olatunji Abubakar Jimoh conceptualized and supervised the work. Hafsat Ololade Okin-Aminu and Olayinka Abosede

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Ojo funded and manage the research, Ayoola Doris Ayodele managed the analysis and wrote the manuscript. Olumuyiwa Joseph Olarotimi managed the bench work and validated experimental protocol. All authors contributed and approved the manuscript for publication.

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