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The Anti-viral and Immunomodulatory Activity of Cinnamon zeylanicum Against "NDV" Newcastle Disease Virus in Chickens

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Abstract

Experiment was conducted to investigate the effects of cinnamon zeylanicum oil as biochemical, immunostimulant and antioxidant activity. One hundred and fifty one day-old chickens were fed by five diet supplements with 0%,0.1%,0.3% of essential oil, and 1%,3% of cinnamon powder for 30 days serum and whole blood were collected for evaluation of T.protein, S.albumin, S.globulin, total antioxidant, lysozyme activity ,phagocytic percent and phagocytic index. The Total .protein showed significant (P-value <0.05)in day 14,21 and 28 while the s.globulin was siginificant at day 14,21 and 28 with (P-value<0.05) when compared with control group while s.albumin showed no-significant ,while total Anti-oxidant capacity (TAC) was high significant (P-value<0.01) at day 14,21 and 28.the challenge test with 10-6 velogenic NDV challenged chicken with mortality (100%) in control group and protection percent (80,86,76 and 50%) in group (2,3,4and 5). Blood phagocytic activity and phagocytic index significantly increased at (P-value<0.01,P-value <0.05) the present investigation showed that cinnamon zeylanicum essential oil and powder exhibits antioxidant ,immunostimmulat and antiviral activity in chickens because of antioxidant dietary supplementary feeding.

Keywords: Cinnamon zeylanicum; NDV;antioxidant activity; immunomodulatory.

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1. Introduction

Newcastle disease is one of the most important avian viral diseases because of its economic impact on the poultry industry. The causative agent, Newcastle disease virus (NDV), is synonymous with avian paramyxovirus type1 [1] It has been classified in the order Mononegavirales, family Paramyxoviridae ,subfamily Paramyxovirinae , and genus Avulavirus [24] NDV infects approximately236 species of pet and free-living birds in addition to domestic avian species (chicken, turkey, goose, duck, and pigeon) [19] Among poultry, chickens are the most susceptible, whereas ducks and geese are the least susceptible. Reference [2] Velogenic NDV is endemic in many countries of Central and South America, the Middle East, and most of Africa and Asia [11]. ND is so virulent birds may die without showing clinical signs [1]) A death rate of almost 100% can occur in unvaccinated poultry flocks. Exotic NDV can infect and cause death even in vaccinated poultry [11]. Cinnamomum zeylanicum blume (family lauraceae) which is popularly known as cinnamon is classified in the botanical division Magnoliophyta, class Magnoliopsida [6]. Cinnamum zeylanicum originates from island of sriLanka (called Ceylon) south east of India cinnamon spice is obtained by drying the central part of the bark and is marketed as quills or powder [6]. Different parts (bark, roots, leaves, flowers, fruit ,stalks, buds) of the plant, cinnamon zeylanicum blume give essential oil with variation in the composition. the active phytochemical constituents of cinnamon are cinnamonaldhyde and eugenol [13]. Cinnamon is herb which is used as a spice in almost all the food preparations. Chemical constituent of cinnamon make it rich in many health beneficial properties like anti-oxidative, antimicrobial, insulin Sensitivity, anti-ulcer, anti-diabetic, anti-inflammatoryetc. Cinnamon bark contains essential oils, resinous compounds, cinnamate, cinnamic acid and cinnamaldehyde[40]reported transcinnamaldehyde,L- borenol, L- bornyl acetate,caryophyllene oxide, eugenol, bcaryophyllene, Enerolidoland cinnamyle acetate. Chemically cinnamon bark also contains terpinolene, - terpinol, - cubebeneand - thujene [40]. This work aims to study the antiviral and immunomodulatory effect of cinnamon powder / cinnamon oil in chickens .

2. Material and Methods

Experimental design: A total of 150 one day old baladi chickens are divided into 5 groups. Table (1) showed the groups fed on cinnamon oil/or powder. Evaluation of total protein ,S.albumin, s.globulin , total antioxidant capacity (TAC), lysozyme activity done at day 7,14,21 and 28. Phagocytic activity and phagocytic index also done, protection percent calculated due to challenge with 106velogenic NDV.

Table 1: showed the groups fed on cinnamon oil/or powder

Group	Vaccination	Oil Treatment	Powder Treatment	Challenged with NDV
Group(1)	-	-	-	(+)
Group(2)	-	0.3+	-	+
Group(3)	-	0.1+	-	+
Group(4)	-	-	3%+	+
Group (5)	-	-	+1%	+

Newcastle Disease virus strain:

NDV isolate was kindly obtained from veterinary serum and vaccine research institute VSVRI, ND department , Abbassia ,Cairo, Egypt and spropagated in allantoic cavity of 9-day old SPF-ECE, and titrated according to [35] was used in challenge test performed after feeding chicken with cinnamon oil &powder for 30 days.

-Cinnamon zeylanicum oil & powder:

Cinnamon quills was purchased from commercial sources and obtained cinnamon oil extract by steam distillation and diluted in sun flower oil with final concentration (0.3%,0.1%) [37].

Cinnamon powder obtained from commercial sources was crushed and thoroughly mixed with ration at final concentration (3% &1%) of diet.

-Challenge test: according to [44]

-Measurement of Phagocytic activity and percentage of Chicken peripheral monocyting using C.albicans:

The test was carried out according to [33] as modified by [19].

-Measurement of Lysozyme activity by agarose cell lyses Assay:

The lysozyme in the serum was measured according to method described by [45].

Determination of Total Antioxidant Capacity:

It used for colormetric determination of total antioxidant capacity serum or plasma; this is to according Koracevic G and his colleagues in 2001.

-Estimation of T.protein:

Purchased from spin-react. It used for Quantative colorimetric determination of T.protein. According [46,42,12].

-Estimation of S.Albumin:

Purchased from spin-react. It used for Quantative colormetric determination of S.Albumin. According to [46,42,12].

-Estimation of s. Globulin:

The proteins of the serum are divide in two fractions, al bumin and globulins [46,12]so,

s. Globulin= T.protein- s.Albumin= g/dl

Statistical analys**is**: data are presented as mean ± SEM. Statistical

Differences among the means of multiple groups were determined by using one-way ANOVA..Calculations were carried out using SPSS. P values P-value < 0.05 (Significant); ****:** P-value < 0.01 (High significant); P-value > 0.05 (Non-significant

3. Results

Table 2: protection percent against challenged with NDV for chickens non-vaccinated with NDV vaccine and fed with cinnamon zeylanicum powder or Cinnamon zeylanicum

Group	No of birds	Challenge test at 30 day post feeding		Protection %
Group 1	30	30	0	0
Group2	30	4	26	86
Group3	30	6	24	80
Group4	30	7	23	76
Group 5	30	15	15	50

The Results showed 86 %protection percent in group 2(while group 3,4 and 5 showed protection(80,76 and 50).while group (1) control group showed 100% mortalities.

SD: Standard deviation; ***:** P-value < 0.05 (Significant); ****:** P-value < 0.01 (High significant); P-value > 0.05 (Non-significant),a,b,c,d e,f,g and e p value is significant between group and anther group

Group(1): control group

Group (2): fed on(0.3 oil of cinnamon zeylanicum) from one day –old

Group(3):): fed on(0.1 oil of cinnamon zeylanicum) from one day –old

Group (4): fed on(3% powder of cinnamon zeylanicum) from one day –old.

Group(5):): fed on(1% powder of cinnamon zeylanicum) from one day –old

t.protein has no significant at day 7 and showed high significant(P-value<0.01) at day14 and significant (P-value <0.05) at day 21,28.in albumin wasn't show any significant at day 7,14 and 21 but had significant (P-value <0.0) at day 28.s.globulin don’t show any significant at day 7, but showed significant (P- value<0.05) at day 14,21 and 28 of feeding.

Table 3: Results of T.protein , S.Albumin,and S.Globulin (g/dl) in non-vaccinated chickens and fed with cinnamon zeylanicum powder or CZ oil

groups	**7 day**			**14 day**			**21 day**			**28 day**			
	T.protein	**s.albumin**	**s.globulin**	**T.protein**	**s.albumin**	**s.globulin**	**T.protein**	**s.albumin**	**s.globulin**	**T.protein**	**s.albumin**	**s. globulin**	
Group (1)	3.2±0.2	1.6±0.3	1.6±0.3	3.3±0.3	1.8±0.2	1.5±0.5	3.3±0.7	1.9±0.4	1.4±0.6	2.8±0.4	1.3±0.2	1.5±0.5	
Group (2)	3.2±1.0	1.9±0.1	1.3±0.9	4.3±0.3^{a}	1.9±0.1	2.6±0.3^{a}	4.7±0.8^{a}	1.6±0.1	2.8±0.9^{a}	3.8±0.2^{a}	1.5±0.0	2.6±0.1^{a}	
Group (3)	3.4±0.3	2.0±0.2	1.4±0.3	4.5±0.6^{a}	2.1±0.3	2.4±0.8^{a}	4.7±0.6^{a}	1.7±0.2	3.1±0.8^{a}	3.3±0.6^{e}	1.5±0.1	1.8±0.6^{f}	
Group (4)	3.3±0.3	2.1±0.7	1.2±0.7	4.2±0.2^{a}	2.1±0.4	2.1±0.2	5.0±0.5^{a}	1.8±0.1	3.2±0.5^{a}	4.0±0.5^{ag}	1.5±0.1	2.5±0.5^{ag}	
Group (5)	3.0±0.3	1.4±0.3^{h}	1.6±0.5	3.3±0.4^{fgh}	2.4±0.1^{af}	0.9±0.5 b^{fgh}	3.4±0.5^{fh}	1.7±0.2	1.6±0.4^{fgh}	2.8±0.3^{fh}	1.9±0.3^{afgh}	1.2±0.4^{fh}	
P value	0.869	0.125	0.841	0.007**	0.092	0.013*	0.015*	0.630	0.019*	0.016*	0.011*	0.017*	

Table 4: Results of total anti-oxidant capacity (mM/L) in non-vaccinated chickens and fed with cinnamon zeylanicum powder or cinnamon zeylanicum oil

Group	Total antioxidant capacity (mM/L)			
	1st week	2nd week	3rd week	4th week
Group (1)	0.1±0.0	0.1±0.1	0.01±0.0	0.1±0.1
Group (2)	0.5±0.3[a]	2.3±0.4[a]	2.4±0.5[a]	2.8±0.1[a]
Group (3)	0.6±0.4[a]	2.0±0.8[a]	2.8±0.1[a]	2.7±0.1[a]
Group (4)	0.3±0.1[g]	1.6±1.0[a]	2.6±0.1[a]	1.4±1.1[afg]
Group (5)	0.2±0.1[g]	0.3±0.1[fgh]	0.2±0.1[fgh]	0.2±0.1[fgh]
P value	0.081	0.003**	0.001**	0.001**

The total anti-oxidant capacity revealed a high significant(P- value<0.01) at day 14,21 and 28 while the values don't show any significant at day 7 of feeding chickens on cinnamon oil/ cinnamon powder.

Table 5: Results of phagocytic activity and phagocytic percent in non-vaccinated chickens and fed with cinnamon zeylanicum powder or cinnamon zeylaNICUM oil

Group	Phagocytic %	Phagocytic index
Group (1) Mean±SD	63.3±4.0	0.2±0.1
Group (2) Mean±SD	89.3±4.6[a]	0.5±0.2
Group (3) Mean±SD	87.3±9.0[a]	0.5±0.1
Group (4) Mean±SD	80.3±2.1[af]	0.4±0.0[a]
Group (5) Mean±SD	79.3±1.5[af]	0.4±0.1[a]
P value	0.001**	0.044*

The phagocytic activity SHOWED high significant (P-value<0.01) (which INgroup 2 and 3 showing (89.3±4.6 and (87.3±9.0) when compared control group (63.3±4.0) . group (4, and 5) showed (80.3±2.1, 79.3±1. 5[),] while PI showed significant (P-value<0.05).

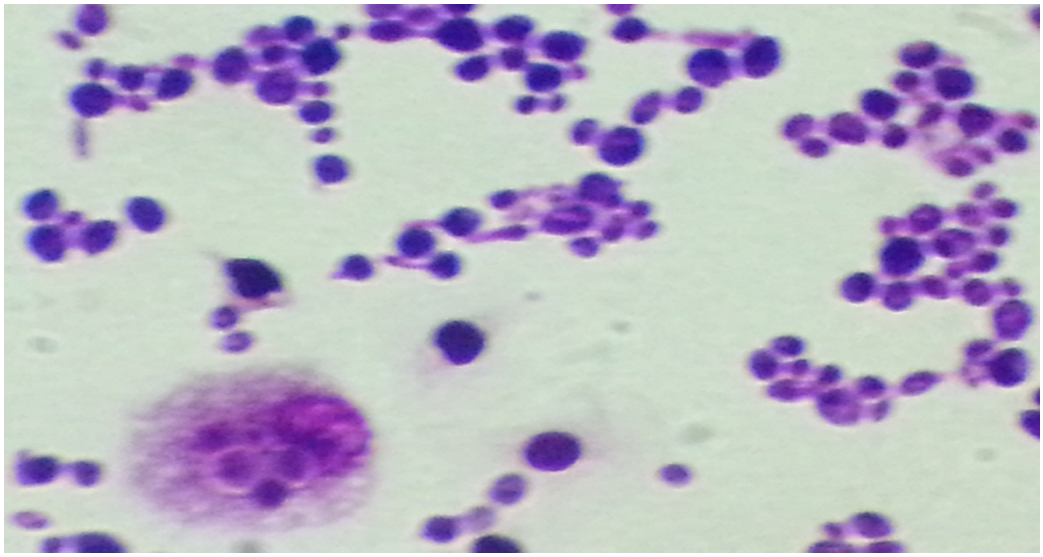

Figure 2: phagocytic cells engulfed candida albicans

Figure: Normal cells and Phagocytic cell engulfed Candida albicans (stained with Giemsa , Image: 100X)

Table 6: Results of Lysozyme activity (µg/ml) non-vaccinated chickens and fed with cinnamon zeylanicum powder or cinnamon Oil

Group	**Lysozyme activity (ug/ml)**			
	7 day	**14 day**	**21 day**	**28 day**
Group (1) Mean±SD	34.2±18.5	56.1±25.4	68.3±45.4	75.6±37.9
Group (2) Mean±SD	56.2±25.5	138.7±67.7[e]	148.5±25.3	94.9±21.1
Group (3) Mean±SD	56.2±25.5	99.9±56.7	153.3±66.2	56.2±25.5[b]
Group (4) Mean±SD	48.9±29.3	112.1±55.2	136.3±46.4	97.4±37.9
Group (5) Mean±SD	27.0±8.7	56.2±25.5	63.4±16.8[cg]	24.5±26.3[bfh]
P value	0.182	0.049*	0.024*	0.103

Lysozyme activity showed significant values (P-value <0.05) at day 14 and 21 while there wasn't any significant at day 7 and 28. The group (2,3 and 4) revealed an increased in mean values at day 14 and 21 (138.7±67.7, 99.9±56.7 and 112.1±55.2) and (148.5±25.3, 153.3±66.2 , 136.3±46.4) when compared control group.

4. Discussion

nowadays natural products such as pure compounds and also plant extract scan provide unlimited opportunities for new antiviral drugs [32]infectious viruses disease has remained important global issues for animal and humans. According to the dependency of viruses on host cells, only a few effect antiviral drugs are available to treat viral disease. Nowadays finding new substances with intercellular and also intracellular antiviral activity is need. These substances must affect viruses without harming the host cell. In ovo injection method was designed because studying the plant in vitro and in vivo is time consuming and expensive (Rezatofighi and his colleagues 2014). In this study, dietary supplementation of different levels of essential oil and powder for their effect on biochemical ,immunological and antioxidant parameters, measurement of the results showed that supplementation of chickens diets with cinnamon essential oil and cinnamon powder for 30 days. On results of T.prorein , S.albumin and S.globulin. The data of effects of dietary supplementation of cinnamon oil concentration and cinnamon powder effect effect on t.protein values in table (3) of chickens showed no significant effect at 1st week and 2nd week ,but revealed a significant at 3rd and 4th week (P-value<0.05) when compared with Negative control. Results proved that cinnamon oil at 0.1 more effective than 0.3 in 2nd and 3rd week ,t.protein had effect when chickens fed on cinnamon powder(3 %) increased at day 21(5.0±0.5) when compared with cinnamon oil at (0.1,0.3). These result agree with [17] that revealed that supplementation with C.verum and Z.officinale increase numerically in T.protein compared to the control. Also, The results agree with [4] who reported that when added 200ppm of essential oil derived from C.verum were added to at protein was observed compare to control group[27] conducted that plasma total protein increase in healthy group and treated groups compared to the untreated diabetic group in study effects cinnamon extract on liver biomarker. The elevated serum T.P levels in spice treated groups may be due to nutritional potential effect of the the treated diets and an increase in body weight gain [24].

The results of measurement S.globulin table (3) showed that supplementation of cinnamon and cinnamon powder had significant effect (P-value<0.05) at day 14,21and 28 when compared with control group. The concentration of cinnamon oil (0.1) and C. powder (3%) had highest values on s.globulin at day21 with (3.1±0.) and (3.1±0.5) when compared witn other groups and control group. (Tolba and his colleagues 2010) found that mixture volatile oils including thyme. oregano , C.verum and capsicum added to two groups of chicks diets at 1 and 2 g/ Kg fed in experimental period which lasted for 12 weeks increased significantly the serum globulin compared to un supplemented control group. It is believed that protective effect of cinnamon verum is the result of combination between the antimicrobial effect of C.verum and stimulated immune system and as result of C.verum administration [17].

On the other hand the cinnamon hadn't any significant effect on serum albumin in table (3)except at day 28. These finding agree with previous studies when spices used as treatment and show no effect on levels of S.albumin [5] Found that rat administration with C.verum orally they showed no significant effect on plasma

albumin concentration compared to rats used as control, other studies suggested increased serum t.Protein, Albumin and Globulin that prevent further accumulation of proteins in kidney tissues resulted in reduction of renal damage. The reduction in albuminurea by cinnamon resulted in improvement of renal function [20]. Cinnamon extract has increased the level of T.protein towards the respective normal value which indicates hepatoprotective activity. Stimulation of Protein synthesis has been advanced as contributory hepatoprotective mechanism which accelerates the regeneration process and production of liver cells[34,38].

Data in table (4) showed the results of Antioxidant activity**:** the effect **of** cinnamon powder /or cinnamon oil of different concentration and proved that cinnamon oil/ powder has Highly significant (P-value <0.01) and has antioxidant activity in serum of chick through 14,21 and 2 8days of feeding . from results the cinnamon oil at (0.1) and cinnamon powder (3%) had high values when compared with control group at day 21and other groups the (1%) cinnamon powder from dietary supplementation has numerically increase when compared with control group. These findings accepted with [14] which revealed that supplementing healthy rats with CBE decreased oxidative damage induced by exhaustive exercise as indicated by MDA serum level and thiol in sup/EX group. Elevated TAC(total antioxidant capacity) for Sup/EX group showing that CBE supplementation increased the amount of antioxidant available to animals. Several studies have indicated that cinnamon has protective effects against many oxidative stress related disease in humans [31]maintained that regular consumption of cinnamon tea decreased the lipid peroxidation and increased TAC in human subjects [26] it was shown that elevation of antioxidant defense in high cholesterol fed rats was subject to cinnamon supplementation . Many studies have reported extracts from bark ,leaves and fruits of cinnamon to have high phenol contents and excellent potential in scavenging free radicals .cinnamon possess anti-oxidant activity in rat fed with high fat diet[15] Cinnamon essential oil has antioxidant properties (Case and his colleagues 1995;lee and his colleagues 2001; Yu and his colleagues 2002; Lee and his colleagues 2007). For studying Immunostimulatory effect of cinnamon data presented in table (2) showed that group (2) fed on cinnamon oil (0.3) with highest protection percent (86%) when compared with control group (0%) and compared other group (3,4 and 5) which showed (80,76 and 50) protection when challenged with 10-6 velogenic NDV strain .

Data presented in table (5) illustrated phagocytic activity and PI)).the phagocytic percent (Phagocytic activity) in chickens fed with cinnamon oil/ or powder high significant (P-value <0.01) the highest phagocytic % of 0.3,0.1 is (89.3±4.6,87.3±9.0) when compared control group while of 3%,1 % revealed (80±2.1/79.3±1.5) when compared control group.PI values d increased significant (P-value<0.05).

The data of lysozyme activity is presented in table (6) was revealed cinnamon oil / or powder dietary supplementation effect on chicken with significant effect(P-value<0.05) an chickens at day 14,21 and 28.

In this study the evaluation immunmodulatory effects of cinnamon zeylanicum oil /or powder against normal and challenged in chickens. The response of cinnamon powder or oil on multiple types of immunity (humor and innate immunity) responses(phagocytosis and lysozymal activity, host resistances mortality) was investigated [8].suggested that PP-CZ (polyphenol) treatments was found to be immunostimulant in multiple arms in immune system in dose dependent manner .PP-CZ treatment increased peripheral PMN and phagocytosis activity in mice, treatment increased the number of resident peritoneal in mice, the results suggest pp-CZ

stimulates non-specific immunity by increasing the number of macrophages and phagocytic activity in mice on sub-acute treatment .there was adose –depedent trend for increased numbers of peritoneal macrophages and increased survival rate in mice .there are specialized phagocytic cells that cells attack foreign substnces ,infectious microbes through destruction and ingestion [30]. Reference [8] demonstrated sub-acute treatment of PP-CZ showed increased peritoneal macrophage and PI.increased PMN by PP-CZ treatment observed in their study indicated potential PP-CZ in stimulating adaptive immunity against infectious pathogens.

Reference [7] demonstrated significant increase in TLC can be considered as indicator for improvement in general resistance, increase in neutrophil in control fed fishes may be non-specific immune respond and increase in lymphocytes counts in cinnamon diet fish can be attribute to specific immune response . cinnamon verum essential oil showed stimulator effect on macrophages, phagocytosis and killing of invading microorganisms by macrophage constitute the body's primary line of defense against infection [41]

5. Conclusion

The present study investigation showed that cinnamon zeylanicum essential oil and powder exhibit significant T.protein , Globulin and albumin and total ant-oxidant activity in chickens. Findings of the study establish cinnamon zeylanicum essential oil and powder had antiviral activity by appreciable immunostimulatory activity by increasing survival percent (challenge test) lysozyme ,PI and phagocytic activity.

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