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Antiviral activity of Curcuma longa against Newcastle disease virus (in vitro and in vivo studies)

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The antiviral activity of *Curcuma longa* against NDV on vero cell culture and in infected chicken was observed. The obtained results showed that low concentration of *Curcuma longa* 0.25% did not produce cytotoxicity on the vero cell. The effect of *Curcuma longa* on NDV titre was studied and the results revealed clearly that chickens treated with 1% *Curcuma longa* as Prophylactic also showed higher protection rate (90%), the chickens that treated with 0.5 % *Curcuma longa* showed protection rate (80%).Chicks that infected with NDV without treatment with *Curcuma longa* showed lower protection rate (8%). The chickens infected then treated with 1% *Curcuma longa* showed protection (65%) and the chickens infected then treated with 0.5% *Curcuma longa* showed protection (40%).

Newcastle disease (ND) is a serious and destructive infectious disease of birds. It is regarded as a major problem facing poultry keeping all over the world whether in large or small scale. Vaccination against NDV is cost and causes some problem. Vaccination with live virus vaccines may play an important role in control of NDV in the infected areas, problems may occur, especially adverse vaccine reactions, such as mild respiratory disease or depressed egg production (Stone, 1997). There is currently a worldwide use of herbal preparations and the active ingredients isolated from medicinal plants in health care. One of these plants is *Curcuma longa* . The dried rhizome of *Curcuma longa*, which has been used for countries as a spice, food preservative and a coloring agent, has been found to be a rich source of beneficial phenolic compounds known as the curcuminoids.The active constituents of turmeric are the flavonoid curcumin and volatile oils including tumerone, atlantone, and zingiberone. Other constituents include sugars, proteins, and resins. The best-researched active constituent is curcumin, which comprises 0.3 to 5.4 percent of raw turmeric (Leung, 1980). So the objective of this work to study the effect of *Curcuma longa* on RNA virus (New castle disease virus) in vivo and in vitro.

Materials and methods

Chicks. A total number of 300 one –day- old chicks were obtained from private hatchery in Beni Suef baladi breeders. They were floor reared, fed on a commercial poultry ration and kept under good hygienic condition throughout the experiment.

***Curcuma Longa* powder.** The powder was purchased from commercial sources and used at concentration 0.25% on vero cells and at concentration 1% and 0.5%, by thoroughly mixed with ration as feed additives.

Biochemical kits. It was used for estimation of total protein and albumin it was purchased from Diamond Company Lot NO 00476544

Detection of *Curcuma longa* cytotoxicity.

Preparation of different concentration of *Curcuma longa* in E 199 medium. In separate vessels 250, 500, 1000, 2000 and 3000 mg of curcuma longa were dissolved in 50 ml of D.W by boiling. Each vessel was completed to 100 ml by addition blank E199 medium .This solution was centrifuged at 1500 rpm for 10 min. and the supernatant was filtrated.

Detection of 50% toxic dose of *Curcuma longa* on Vero cells. In 96 tissue culture plate (96 wells) with confluent monolayer of vero cells ,different concentration of *Curcuma longa* were added each concentration / each row of the microtitre plate as follows 0.25, 0.5, 1,2,3% in row A, B, C, D, E and the last three rows were kept untreated as cell control . The plats were incubated at 37°C and 5% Co_2 and 80% humidity for about 6 days with daily examination for the presence of cytopathic effects.

Cytopathogencity of NDV treated with *Curcuma longa* on Vero cells. It was done according to (Sebla *et al.*, 2007). Flat bottom micro titer plat contained 90% confluence of Vero cells was used. The medium were poured off in 24-wells and 100 µl of maintenance medium was added to each well. In each well of the first row 50 µl of $10^7$of Newcastle disease (virus) were added

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Table (1): Treatment and infection of chicks used in this study.

Group	*Curcuma longa*	NDV infection	NO. of chicks
G1- infected not treated	-	+	50
G - treated with 1% then infected	+1%	+	50
G3- treated with 0.5% then infected	+0.5%	+	50
G4 – infected then treated with 1%	+1%	+	50
G5 – infected then treated with 0.5%	+0.5%	+	50
G6- control treated	+1%	-	25
G7 - non infected non treated	-	-	25

and the second row 50 µl of 0.25% concentration of *Curcuma longa* (curcuma) were added.

In the third row 50 µl of 10^7 of Newcastle disease virus and 50 µl of *Curcuma longa* (virus +*Curcuma longa*) were added while in the forth row only 50 µl of maintenance media (control) were added. The plate was then incubated in CO2 incubator at 37°C and 5% CO2 with daily observation for cytopathic effect till 120 hrs.

Detection the effect of *Curcuma longa* on Newcastle disease virus in different times on Vero cell for cell free and cell associated virus. In healthy vero cells with 80-90% confluence 100 µl of NDV of 7 log 10 TCID50 and 100 µl of *Curcuma longa* at concentration 0.25% were added. The plates were incubated in CO2 incubator at 37°C and CO2 5%. Cell free and cell associated virus was harvested after 10 min., 20 min. , 40 min., 1h , 2h, 6h, 12 h, 24 h, 48 h, 72 h, 96h and 120 h after incubations and titrated according to (Reed and Munch, 1938).

Experimental design. A total number of three hundred chicks one day old were used in this studies. The used chicks were divided into seven separate groups and treated as in (Table 1). Group (1) Fed commercial ration from one- day old till day of infection with NDV. Group (2) Fed commercial ration contained of curcuma longa, from one day till infection with VV NDV. Group (3) Fed commercial ration contained 0.5 % *Curcuma longa* from one–day old till infection with VV NDV.

Group (4) Fed commercial ration from one- day old till infection with VV NDV, than fed on ration contained 1 % of commercial *Curcuma longa* (as treatment). Group (5) Fed commercial ration from one- day old till infection with VV NDV, than fed on ration contained 0.5% of commercial *Curcuma longa* (treatment).| Group (6) Fed commercial ration contained 1% of commercial *Curcuma longa* and not infected. Group (7) Fed commercial ration and not infected (control negative). Blood samples were taken from chicks of each group at age 7, 10, 12 and 15 day old to detect the level of maternal HI antibodies to NDV. After withdrawal of maternal Abs, Group 1, group 2, group 3, group 4 and group 5 had been infected with VVNDV by a dose of 0.5 ml /bird containing 10^6 EID_{50} (I/M), infected chickens were observed daily and clinical signs were recorded. The number of dead and live chicks was recorded. Blood sample were taken from live chicks and their sera were separated and tested by HI test.

Haemagglutination test (HA) and Haemagglution inhibition test. They were done according to the slandered procedure by (Anon, 1971).

Determination of total protein, albumin and globulin. Total protein, albumen and globulin were performed according to (Burtis, 1999) while A/G ratio was performed according to (Joce, 1999).

Results and Discussion

Newcastle disease (ND) is a highly contagious and fatal destructive disease, which attacks chiefly chickens and turkeys usually in acute form, but some time in sub acute or chronic form, This virus belongs to the genus Avulavirus in the family *Paramyxoviridae* (Houston, 2002).

During the last 25 years, there have been numerous broad-based screening programmes initiated in different parts of the globe to evaluate the antiviral activity of medicinal plants for in vitro and in vivo assays.

Many traditional medicinal plants have been reported to have strong antiviral activity and some of them have already been used to treat animals and people who suffer from viral infection (Hudson, 1990;Venkateswaran *et al.*, 1987; Thyagarajan *et al.*, 1988, 1990). This work aims to study the effect of cheep and natural herbal medicinal plant, known as *Curcuma longa*, on NDV in infected cell culture and chicken.

Turmeric consists of 3-5% curcuminoids. Curcumin which is the most important fraction responsible for the biological activities of turmeric.

Table (2): Detection of 50% toxic dose of *Curcuma longa* on vero cells.

Conc. Of plant % mg/100 ml	Grade of CPE			
	After 24 hrs	After 48 hrs	After 72 hrs	After 96 hrs
0.25	-	-	-	-
0.5	-	-	+	+
1	-	+	++	++
2	+	+	++	++
3	+	+	++	+++

Table (3): The effect of *Curcuma longa* on Newcastle disease virus at different time interval.

Hours of infection	Intensity of CPE of infected, infected and treated cells				vero cells infected with NDV and treated with *Curcuma longa*			
	+	++	++++	++++	+	++	+++	++++
12	•				°	°	°	°
24		••			°	°	°	°
48			•••		°	°	°	°
72			•••		°	°	°	°
96				••••	°	°	°	°
120				••••	°	°	°	°

° :- no cytopatheic effect on Vero cell
• :- showing focal rounding and shrinkage of the infected cells
•• :- Beginning of cytoplasmic fusion which results in syncytial formation
••• :- Large cytoplasmic vacuoles develop in the multinucleated cells or syncytia (poly-karyons)
•••• :- Cell detachment and destruction of cells

Table (4): Titration of Newcastle diseases virus + curcuma longa 0.25% on Vero cells.

Time	Virus titer (log_{10} TCID50 /ml)			
	Cell free virus (CFV) log_{10} reduction		Cell associated virus (CAV) log_{10} reduction	
0 Min.	7	0	0	0
10 Min.	5	2	0	7
20 Min.	5	2	0	7
40 Min.	4	3	2	5
1 h	2	5	4	3
2 h	1	6	5	2
6h	1	6	2	5
12h	0	7	2	5
24h	0	7	2	5
48h	0	7	2	5
72h	0	7	0	7
96 h	0	7	0	7
120 h	0	7	0	7

Table (5): Immunoflourscent test.

Hours of infection	Intensity of fluorescent in Vero cells infected with NDV and treated with *Curcuma longa*	Intensity of fluorescent in Vero cell infected with NDV
1	±	±
3	+	+
6	-	+++
12	-	+++
24	-	+++
48	-	+++
72	-	++
96	-	++
120	-	++

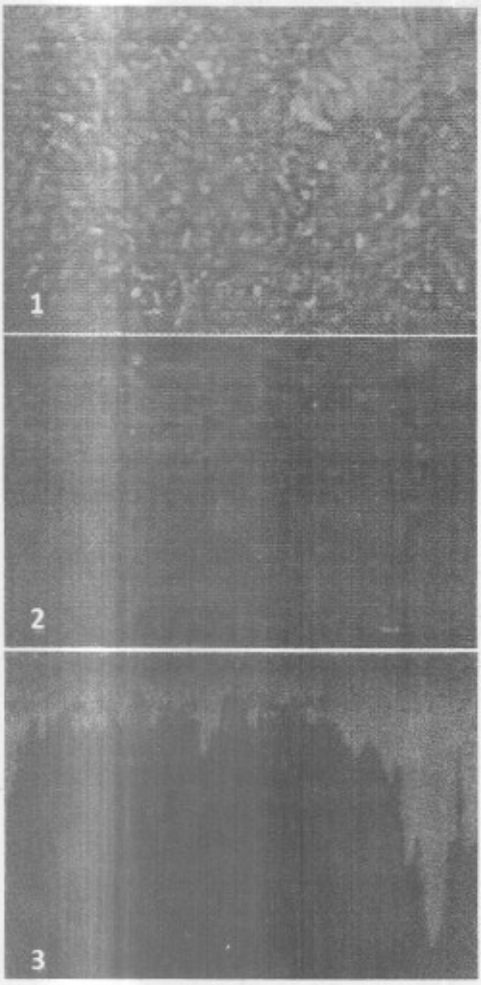

Fig.(1): Vero cell infected with NDV and stained with fluorescent showed fluorescent foci after 24 h (X200).
Fig.(2): Vero cell infected with NDV and treated with *Curcuma longa* showed small fluorescent foci after 3h (X200).
Fig.(3): Vero cell infected with NDV and treated with *Curcuma longa* stained with fluorescent after 24h (X200).

The active constituents of turmeric are the flavonoid curcumin and volatile oils including tumerone, atlantone, and zingiberone. Other constituents include sugars, proteins, and resins. The best-researched active constituent is curcumin, which comprises 0.3 to 5.4 percent of raw turmeric (Leung, 1980).

Curcumin (diferuloylmethane), is a natural polyphenolic compound extracted from the spice turmeric, has been reported to have anti-inflammatory, antioxidant, and anti proliferative properties by modulating multiple cellular machineries. It inhibits several intracellular signaling pathways, including the mitogen-activated protein kinases (MAPKs) and casein kinase II (CKII), in various cell types (Subhash *et al.*, 2007).

In this study, the efficacy of curcuma longa as natural antiviral product was estimated by using RNA virus model (NDV). For studying the antiviral activity of *Curcuma longa*, firstly we determine its toxicity on Vero cell culture using different concentration of the plant. Data presented in (Table 2) showed that low concentration of *Curcuma longa* 0.25% did not induce any CPE on vero cells culture up to 96 h PI while the concentration of 0.5% and 1% did not induce CPE in the first 24 h but death of cells was appeared after that denoting to the toxic effect of *Curcuma longa* in using these concentrations. Therefore we used low concentration of *Curcuma longa* 0.25% to evaluate its antiviral activity against NDV on Vero cells.

The antiviral activity of *Curcuma longa* on NDV was detected either qualitatively or quantitatively. Data presented in (Table 3) showed that *Curcuma longa* in concentration 0.25% has the ability to prevent cytopathogencity of NDV on vero cells after 12 h till 120 h PI.

For quantitative estimation of this effect, data presented in (Table 4) showed the result of titration of NDV in CFV and CAV when treated with 0.25% *Curcuma longa*. *Curcuma longa* inhibited the replication of NDV as the infectivity titer of the virus was decreased 2 log $_{10}$ 2 hrs post infected (CAV) and 5 log $_{10}$ at 6 hrs post infected (CAV) till 48 h post infection then decreased 7 log 10 after 72-120 h post infection . The cell free virus (CFV) didn't found in tissue culture medium after 12 h post infection. The absence of CFV in treated sample 12 h PI and no CAV was detected 72 h PI beside the decrease in NDV infectivity titers may be attributed to the action of curcuma that strongly reduced viral RNA expression or synthesis of viral proteins. The inhibitory effect of *Curcuma longa* may be due to the inhibition of replication of viral genome, protein synthesis, virus assembly, virus release and virus titer and these results agree with (Xiaoning *et al.*, 2007) who found that treatment with curcumin significantly

Table (6): The effect of *Curcuma longa* on chicken infected with Newcastle virus.

group	Number of bird examined	Week post treatment								protection %
		1st week		2nd week		3rd week		4th week		
		live	dead	live	dead	live	dead	live	dead	
1	50	20	30	10	10	6	4	4	2	8
2	50	50	0	47	3	45	2	45	0	90
3	50	45	5	40	5	40	0	40	0	80
4	50	35	15	30	5	28	2	28	0	56
5	50	30	20	27	3	25	5	20	0	40
6	25	25	0	25	0	25	0	25	0	100
7	25	25	0	25	0	25	0	25	0	100

Table (7): Geometric Mean of NDV HI antibody titers on different groups.

Groups of chicks	Geometric mean of HI antibody titers per weeks after challenge			
	1	2	3	4
1	128	1024	4096	512
2	32	16	8	8
3	64	32	16	16
4	128	64	32	16
5	128	64	64	32
6	0	0	0	0
7	0	0	0	0

Table (8): The effect of *Curcuma longa* on total protein, albumin and globulin of treated chicks.

Week	Parameter	Group 1	Group 2	Group 3	Group 4	Group 5	Group 6	Group7
1st week	T.P.	2.76	3.36	3.08	2.88	2.84	3.6	3.2
	Albumin	1.06	1.92	1.8	1.4	1.4	1.96	1.78
	Globulin	1.69	1.44	1.28	1.52	1.4	1.6	144
	A.G.ratio	0.62	1.32	1.25	0.8	0.94	1.22	1.094
2nd week	T.P.	2.64	3.64	3.56	3.04	3	3.64	3.2
	Albumin	0.84	1.8	1.8	1.44	1.44	2.04	1.7
	Globulin	1.8	1.84	1.8	1.52	1.48	1.6	1.48
	A.G.ratio	0.4	1.1	1.1	0.92	0.9	1.2	1
3rd week	T.P.	2. 4	3.76	3.72	3.32	3.2	4.08	3.16
	Albumin	0.64	1.92	1.92	1.76	1.76	2.12	1.64
	Globulin	1.84	1.84	1.8	1.52	1.6	1.96	1.56
	AG ratio	0.3	1.04	1.06	1.1	1.1	1.08	1.02
4th week	T.P.	2.44	3.76	3.72	3.36	3.2	4.14	3.2
	Albumin	0.95	1.92	1.84	1.76	1.76	2.12	1.72
	Globulin	1 .22	1.84	1.8	1.6	1.6	2	1.6
	A/G ratio	0.8	1.04	1.06	1.1	1.1	1.06	1.01

reduced viral RNA expression, protein synthesis, and virus titer and protected cells from virus-induced cytopathic effect and apoptosis. This result was confirmed by IFA test on Vero cell culture (Table 5, Fig.1-3). The inhibition of NDV proteins may be due to either prevention of viral protein synthesis or prevention of ribosomal activities (Xiaoning *et al.*, 2007). Thus, our results suggest an important antiviral effect of *Curcuma longa* wherein it potently inhibits replication of NDV and accumulation of its proteins as detected by IFA during comparing the infected and treated cells with curcuma Longa with infected untreated cells.

An experimental design for studying the antiviral activities of *Curcuma longa* against NDV in Vivo was planned .A total number of three hundred chicks one day old were used in this studies and the date presented in (Table 6) revealed clearly that that chicks treated and infected with 1% *Curcuma longa* as a prophylactic dose showed higher protection rate (90%) while the chicks that treated with 0.5 % and infected showed protection rate (80%). Chicks that infected without treatment with *Curcuma longa* showed lower protection rate (8%). The chicks infected then treated with 1% *Curcuma longa* as treatment dose showed protection

(65%) and the chicks infected then treated with 0.5% *Curcuma longa* showed protection(40 %), while chicks infected and not treated showed lowest protection (8 %) as 92% of the infected chicken were died. Theses results are in agreement with (Sebla *et al.*, 2007) who found that curcumin inhibits herpes simplex virus. Hye *et al.* (2009) also found that *Curcuma longa* extract has antiviral effect against hepatitis B virus replication. And the result where confirmed by HI titer in (Table 7). It is very clear that the NDV HI antibody titers increased gradually from the first week till 3rd week (maximum value) and then decreased gradually.

On measuring total protein , albumin and globulin fraction in sera of different chicken groups data present in (Table 8) showed higher total serum protein 3.72 in *Curcuma longa* treated group than other groups, also significant higher globulin fraction 1.84, and 1.8 in curcuma longa treated group than other non treated while group 1 that infected with NDV without treatment showed decrease in both total protein and albumin .This result agree with (Shabon, 2007) who recorded that curcuma was found to elevate the protein profile total protein, albumin and globulin.

From all previous discussed data we could conclude the anti-NDV activity of *Curcuma longa* and we recommended the use of *Curcuma longa* as feed additives for its antiviral activity and immune stimulating effect.

References

Anom, (1971): Methods for examining poultry biologics and for identifying & quantifying avian pathogens. National Academy of Science Wahington D.C.

Burtis, A. (1999): Tietz Texbook of clinical chemistry . 3rd ed AACC.

Houston, (2002): Over expression of NDV protein enhances Arch Virol., 147(1):1071-1076.

Hudson, J. B. (1990): Antiviral Compounds from Plants. nBoca Raton, Ann Arbor, Boston.

Hye, J. K.; Hwa, S. Y.; Jin, C. K.; Chan, S. P.; Mi, S. C.; Mijee, K.; Hyangsoon, C.; Jung, S. M.; Yong, S. K.; Seong, W. Y. and Jeong K. A. (2009): Antiviral effect of Curcuma longa Linn extract against hepatitis B virus replication([a]Department of Microbiology, Chungnam National Univ., Daejeon 305-764, South Koreaprotease. Phytotherapy Res., 14:510– 516.

Joce, L. K. (1999): laboratory diagnostic tests with nursing impacation 5th ed.

Leung, A. (1980): Encyclopedia of Common Natural Ingredients Used in Food, Drugs, and Cosmetics. New York, NY: John Wiley 313-314.

Reed L. J. and Muench, H. (1938): Simple method of estimating fifty precent end points. Am. J. Hyg., 27:493-497.

Sebla, B. K.; James, D.; Martha, E. R. and Steven J. T. (2007): Curcumin inhibits herpes simplex virus immediate-early gene expression by a mechanism independent of p300/CBP histone acetyltransferase activity. 373(2):239-247.

Shabon, M. H. (2007): Radioorotective effect of curcuma. National Centre for Nuclear Safety and Radiation Control, Atomic Energy Authority, Cairo, Egypt.

Stone, H. D.; Mitchell, B. and Brugh, M. (1997): In ovo vaccination ofchicken embryos with experimental Newcastle disease and avian influenza oil – emulsion vaccines . Avian Dis., 41(4):856.

Subhash, C. G.; Xiaohua, G. and Scott, D. (2007): Immunodulation by curcumin 10.1007/978-0-387-4640-5-14

Thyagarajan, S. P.; Subramanian, S.; Thiranapasundaru, T.; Venkateswaran, P. S. and Blumberg, B. S. (1988): Effect of Phyllanthus amarus on chronic carriers of hepatitis B virus. Lancet, 2:764-766.

Thyagarajan, S. P.; Subramanian, S.; Thiranapas-undaru, T.; Thyagarajan, S. P.; Jayaram, S. and Valliammai, T. (1990): *Phyllanthus amarus* and hepatitis B (Letter). Lancet, 336:49-50.

Venkateswaran, P. S.; Millman, I. and Blumberg, B. S. (1987): Effects of an extract from Phyllanthus niruri on hepatitis B and Woodchuck hepatitis viruses: in vitro and in vivo studies. Proceedings of the National Academy of Sciences of the USA 84, 274– 278.

Xiaoning, S.; Yahong, W.; Jerry Wong, Jingchun Z.; Bruce, M. M. and Honglin, L. (2007): Curcumin inhibits replication of CVB3.

التاثير المضاد للفيروسات للكركم ضد فيروس النيوكاسل (دراسة معملية وحقلية)

أجريت هذه الدراسة لتقييم إحتمالية وجود تأثير مثبط للكركم على فيروس النيوكاسل على مستويين أولاً على المستوى المعملي باستخدام خلايا الفيرو ثانياً على مستوى الكائن الحي باستخدام الكتاكيت وتم تجريب عدة جرعات من الكركم على الخلايا وهى ٢٥٠ مجم و٥٠٠ مجم و ١ جم و ٢ جم و ٣ جم وجد أن الجرعة من الكركم التى لا تسبب موت الخلايا هى ٢٥٠ مجم بعد مرور ٤ أيام من الحقن لذلك تم اختيار هذه الجرعة ثم بعد ذلك تم معالجة الخلايا المصابة بالفيرس بالكركم ووجد أن الكركم استطاع أن يحمى الخلايا من الإصابة بالفيروس وتم قياس القوة العيارية للفيرس المعالج بالكركم داخل وخارج الخلايا ووجد أن الكركم استطاع أن يقلل القوة العيارية للفيرس بعد دخوله إلى الخلايا عن طريق التدخل فى عملية تكاثر الفيروس داخل الخلايا. أما على مستوى الكائن الحى فقد أظهرت النتائج أن الكركم له قدرة على حماية الكتاكيت من الموت بسبب الفيرس حيث تصل نسبة الحماية للمجموعات الوقائية إلى ٩٠% و٨٠% للجرعة ١% و ٥,٠% على التوالى بينما تصل نسبة الحماية للمجموعات المعالجة الى ٥٦% و٤٠% للجرعة ١% و ٥,٠% على التوالى كما وجد أن الكركم له القدرة على رفع نسبة البروتين بينما الفيروس له القدرة على تقليله.