Effect of Force 6® Poultry on Infectious Bursal disease Virus in *Vitro*

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Summary

The aim of this study was to investigate the effect of force 6 poultry (conc.) in Log. of infectious bursal disease Virus on tissue culture and Virus, on Virus alone and on tissue culture alone. Different concentrations (0.5, 12.5, 25 and 50 µg/ml) were used to consider the anti-viral activity. The result showed effect of force 6® poultry (conc.) in Log. of Infectious Bursal Disease Virus. On tissue culture and Virus, the results revealed higher value with more significant ($P<0.05$) differences at concentration (0.5 µg/ml) than other concentrations and control; On Virus alone, it showed more significant ($P<0.05$) differences at concentration (0.5 µg/ml) than concentration (50 µg/ml) which showed less value of Virus growth. And on tissue culture alone (chicken embryo fibroblast) it showed lesser value and significant ($P<0.05$) differences than other concentrations. In conclusion, the main changes in tissue culture explained at concentration (0.5, 25 and 50 µg/ml) but not (12.5 µg/ml and control). This group also were more affected on Virus titer when compared with other than two groups (tissue culture and Virus and Virus alone).

Keywords: Force6®Poultry, Infectious Bursal disease Virus, Virus.

Introduction

Force 6® Poultry (curcumin) is an innovative and patented premixture of feed additives ensuring the controlled release of active component curcumin in a bio-available form. Force 6 Poultry is a performances booster designed for high productive animals. The expected effects are an improvement of global performances thank an improvement of immunity and a limitation of anti-inflammatory process and oxidative stress which orient metabolism into a protection of organism instead of growth and feed efficiency (1).

The Curcumin (*Curcuma longa*) plant, a perennial herb belonging to the ginger family, is cultivated extensively in south and southeast tropical Asia. The rhizome of this plant is also referred to as the "root" and is the most useful part of the plant for culinary and medicinal purposes. The most active component of turmeric is curcumin, which makes up 2 to 5% of the spice (2). Curcumin is currently undergoing clinical trials for AIDS patients and its effect has been determined on purified human. These observations suggest new strategies for antiviral drug development based upon curcumin as a lead compound for the development of inhibitors of HIV-1 integrase (3). Curcuma longa extract can be used as a safe and specific drug for patients with liver diseases caused by HBV infection (4). Also curcumin had a direct effect on viral particle infectivity that was reflected by inhibition of heamagglutination (5). This study has been conducted to explore the compatibility of this product as antiviral activity on status of broiler chicks. Infectious Bursal disease Virus (IBDV) have been a widely and severe immune suppressive agent, as well as an oxidative agent (6). So in this study it used Force 6® Poultry in IBDV vaccinated and challenged broiler chicks.

Materials and Methods

Composition of force 6® Poultry is: Technological additive: 1g binder, anticaking-1: Silicic acid precipitated and dried ,fatty acid esterified with glycerol, glycerin, water, flavoring substance (natural), Technological additive: 3b compounds of trace elements: zinc sulphate hepathydrate,1b-antioxidant: sodium ascorbate, (PHODE-company-france) the experiment was worked in (JOVAC company - Jordan) (2013). To prepare aqueous extracts, (0.5, 12.5, 25 and 50) µg/ml of each dry powdered curcumin doses were infused in distilled water until complete exhaustion (usually for 72 hr). The extract was then filtered using muslin or Whatman paper No.1. (6). Then mono layer of cells of SPF/ chicken embryo fibroblast grown in 96-micro well

plates for 24 h were washed twice with PBS. Then force 6® Poultry was inoculated using the following preparation.

A: Curcumin mixed with Virus: This was prepared 1 ml of extract curcumin concentrations (0.5, 12.5, 25 and 50) each separately and then mixed with the Virus and incubated for (1hr.) at 37°C. Then it was inoculated in primary chicken embryo cell (CEF) mono layer in one flask (25cm^2) to each concentration. Development of cytopathic effects was observed after every 24 hours interval for five days. Then the Virus titer was conducted to demonstrate the level of growth of various concentrations.

B: Curcumin mixed with Tissue culture (pre-inoculation of Virus): This was prepared as 1 ml of extract curcumin concentrations (0.5, 12.5, 25 and 50) each separately was added to CEF, incubated for (1hr.) at 37°C. Then the mono layer was washed and was inoculated with (0.5 ml) of stock Virus and incubated for (1hr.) at 37ºC in one flask (25cm^2) to each concentration. Development of cytopathic effects was observed after every 24 hours interval for five days. Then was freezed and thawed 3 times for each concentration and titration of the Virus conducted to demonstrate the level of growth of various concentrations.

C: Curcumin mixed with Virus and tissue culture (post-inoculation of Virus): Curcumin at concentrations (0.5, 12.5, 25 and 50) were prepared in maintenance media. (0.5 ml) of Virus was inoculated in CEF and incubated for (1hr.) at 37ºC in one flask (25cm^2) to each concentration. Then maintenance media containing curcumin with different concentrations was added. The Statistical Analysis System-SAS (2010) (7) was used to explain the effect of different factors in study's parameters. Chi-square test was used to compare between percentage and least significant difference-LSD and Duncan multiple range test was used to compare between means in this study.

Results and Discussion

The result in (Table, 1) showed that concentration (0.5 µg/ml) showed higher value and was (7.9 TCID50) with more significant (P<0.05) differences than the concentration (50 µg/ml) which was (7.3 TCID50).

On Virus: The result showed that concentration (0.5 µg/ml) (7.7 TCID50) shows more significant (P<0.05) differences than with concentration (50 µg/ml) which showed less value of Virus growth (7.3 TCID50), but the groups (control, 12.5, 25 µg/ml) showed no differences between them (Table, 1).

On tissue culture: The result showed that concentration 50 µg/ml was (6.7 TCID50) and showed lesser value and significant (P<0.05) differences than others concentrations. Also at concentration 25 µg/ml it showed no differences than others concentration (Table, 1).

The result in (row): (Control) showed no difference between groups. At concentration (0.5µg/ml), it showed a significant (P<0.05) differences between tissue culture group with lesser value (7.5 TCID50) and tissue culture and Virus group with higher value (7.9 TCID50), in Virus group there was no difference. At concentration (12.5µg/ml) there was no difference among groups. At concentration (25µg/ml) it showed significant (P<0.05) differences among (tissue culture) group with less value (7.0 TCID50) and other groups at value (7.5 TCID50). Finally at concentration (50 µg/ml) it showed significant (P<0.05) differences among (tissue culture) group with less value (6.7 TCID50) and (tissue culture and Virus) and (Virus) groups at value (7.3 - 7.2 TCID50) respectively, in **(**Table, 1 and Fig. 1).

The current result shows the effect of Force 6® Poultry on Tissue culture and Virus only concentration (0.5µg/ml) which increase Virus titer and other concentration not negligible difference due to the curcumin not effect in all doses on tissue culture and Virus together, this mean that no effect on Virus replication in tissue culture. This result was in agreement with (1) showed Madin-Darby canine kidney (MDCK) cells were treated with curcumin at the indicated concentrations and infected with Virus showed no significant difference due to effect of (MEM) on biological properties of curumin. Also the result of Force 6® Poultry on Virus showes that concentration (50 µg/ml) was significant difference due to effect of curcumin on concentration (0.5 µg/ml) but not

on other concentrations. The evidence of the present study suggested that the curcumin had no direct effect on Virus because the Birna Virus (IBDV) was non envelope. This agrees with (8).

This study demonstrates a novel mechanism by which curcumin inhibits the infectivity of enveloped Viruses. In all analyzed enveloped Viruses, including the influenza Virus, curcumin inhibited plaque formation. In contrast, the non-enveloped entero Virus 71 remained unaffected by curcumin treatment.

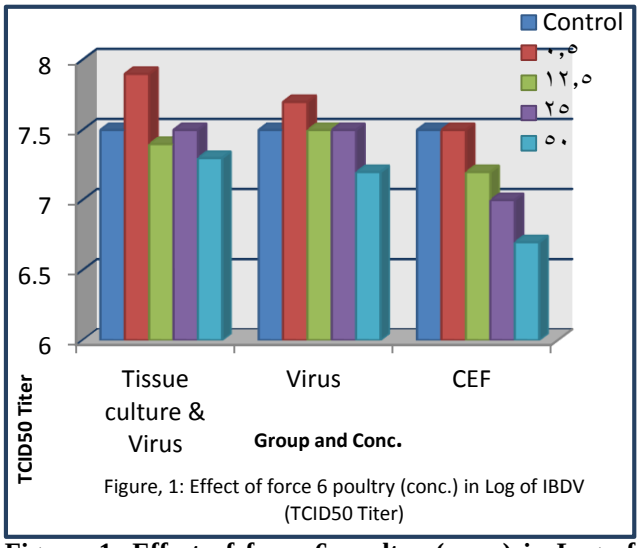
Also the results showed that curcumin pretreatment (at concentrations of 31.2 µM or higher) inhibited the binding of Newcastle disease Virus (NDV) to chicken RBCs, as indicated by the spot-like appearance of non-hemagglutinated cells. Also (5) demonstrated that curcumin had a direct effect on viral particle infectivity that was reflected by the inhibition of haemagglutination. This effect was observed in H1N1 as well as in H6N1 subtype. As well as Curcumin is known to inhibit the immediate early gene promoters of herpes simplex Virus type 1 (HSV-1) (9). In addition aqueous extract of Curcuma longa Linn (CLL) was prepared and used to analyze its antiviral activity against hepatitis B Virus (HBV) replication. CLL extract can be used as a safe and specific drug for patients with liver diseases caused by HBV infection (4). The evidence of the present study suggested that the main changes in Tissue Culture is explained at concentration (0.5, 25, 50 µg/ml) but not (12.5 µg/ml and control). This group also more affected Virus titer when compared with other than two groups (Tissue Culture and Virus and Virus) because curcumin had direct effect on tissue culture and this result was in agreement with other references (Table, 1). Intracellular localization of curcumin was monitored and the results indicated that curcumin is located both in the cell membrane and the nucleus. Subcellular fractionation of curcumin was loaded cells supported the differential distribution of curcumin in membrane, cytoplasm and nuclear (10). As well as (11) that curcumin strikingly modulates protein of iron metabolism in cell and tissues, suggesting that curcumin has properties of an iron chelator.

In conclusion, Study demonstrates the effect of curcumin on Virus growth (IBDV) and there are no previous studies on this Virus in poultry diseases.

Table, 1: Effect of force 6 poultry (conc.) in Log of IBDV on Tissue Culture and Virus – Virus – Tissue Culture. (TCID50 Titer).

Force 6 poultry conc. (µg/ml)	Tissue culture and Virus/post inoculation	Virus	CEF Pre inoculation	LSD Value
Control	7.5 b	7.5 ab	7.5 a	0.377 NS
0.5	7.9 a	7.7 a	7.5 a	0.362*
12.5	7.4 b	7.5 ab	7.2 a	0.392 NS
25	7.5 b	7.5 ab	7.0 ab	0.386 *
50	7.3 b	7.2 b	6.7 b	0.402 *
LSD Value	0.355 *	0.301*	0.419 *	

Different letter: Significant (P<0.05) difference between means in column (small letter) and in row (big letter)
Medium that carries two characters (ab) mean does not differentiate from (a) nor (b).



Figure, 1: Effect of force 6 poultry (conc.) in Log of IBDV (TCID50 Titer)

Figure, 1: Effect of force 6 poultry (conc.) in Log of IBDV (TCID50 Titer)

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تأثير القوة 6® للدواجن في فايروس التهاب الجراب المعدي في المختبر

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الخلاصة

هدفت الدراسة لمعرفة تأثير القوة 6 للدواجن في تراكيز لوغاريتمية لفايروس التهاب الجراب المعدي في خلايا الزرع النسجي والفايروس وفي الفايروس لوحده وفي خلايا الزرع النسجي لوحدها (التأثير الخلوي). استعملت تراكيز مختلفة (0.5 و12.5 و25 و50 مايكروغرام/ مل) بإعتباره له فعالية كمضاد فيروسي. أظهرت نتائج تأثيرالقوة 6 للدواجن بتراكيز لوغاريتمية لفايروس التهاب الجراب المعدي في خلايا الزرع النسجي والفايروس فرق معنوي عند مستوى ($P<0.05$) بتركيز (0.5 ميكروغرام/ مل) والذي سجل أعلى قيمة بالمقارنة مع باقي التراكيز الأخرى ومجموعة السيطرة. أما في الفايروس منفرد فقد أظهرت النتائج فرق معنوي عند مستوى ($P<0.05$) بتركيز(0.5 ميكروغرام/ مل) بالمقارنة مع تركيز (50 ميكروغرام/ مل) الذي أظهر أقل قيمة لنمو الفايروس. أما خلايا الزرع النسجي منفرداً فقد أظهرت فرق معنوي عند مستوى ($P<0.05$) بتركيز (50 ميكروغرام/ مل) مقارنة مع التراكيز الأخرى ومجموعة السيطرة. تستنتج الدراسة أنّ التغير الرئيس قد حصل في خلايا الزرع النسجي وفي تراكيز(0.5 و25 و50 ميكروغرام/ مل) ولم يحصل بتركيز (12.5 ميكروغرام/ مل) ومجموعة السيطرة. كذلك فإن مجموعة (خلايا الزرع النسجي) كان لها التأثير الأكبر في معيارية الفايروس مقارنة بالمجموعتين الآخرى (في خلايا الزرع النسجي والفايروس وفي الفايروس منفرد).

الكلمات المفتاحية: القوة 6® للدواجن، فايروس التهاب الجراب المعدي، فيروس.