

## **The Effects of Levamisole Hydrochloride and LaSota Strain of Newcastle Disease Virus in the Treatment of Cancer**

تأثير هيدروكلوريد الليفاميزول وعترة لاسوتا لفايروس نيوكاسل في علاج السرطان

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### **Abstract:**

This study concluded the effects of levamisole hydrochloride and LaSota strain of Newcastle disease virus in the cancer treatment using seven groups of mice . The first group , which was treated with levamisole hydrochloride orally and multiple injection of LaSota strain of Newcastle disease virus (LS. NDV) Intratumoral, show reduction in the relative tumor volume (R.T.V) and the percentage at the end of treatment (33%) statistically significant compared with the tumor size before treatment, that means (67%) of tumor size was regression. While the relative tumor volume in the group treated with levamisole orally and injection of virus intraperitoneally and group treated with levamisole only show increased in the R.T.V but less than increased will occur in the positive and negative control groups.

The relative body weight show increased in the group treated with LS. NDV intratumoral but decrease in the group treated intraperitoneally compared with the body weight before start the treatment.

### **الخلاصة :**

اشتملت هذه الدراسة على تأثير كل من هيدروكلوريد الليفاميزول وسلالة لاسوتا لفايروس نيوكاسل في علاج السرطان باستخدام سبع من الفئران المختبرية . أظهرت المجموعة الاولى والمعالجة بالليفاميزول عن طريق الفم والحقن المتعدد من عترة لاسوتا لفايروس نيوكاسل داخل كتلة الورم انخفاض في حجم الورم النسبي وبلغت النسبة المئوية في نهاية التجربة (33%) وبشكل مهم احصائي مقارنة مع حجم الورم قبل بدء العلاج وهذا يدل على ان (67%) من حجم الورم الأصلي تقهقر. بينما أظهرت المجموعة المعالجة بالليفاميزول والحقن المتعدد لعترة لاسوتا داخل تجويف الخلب وكذلك المجموعة المعالجة بالليفاميزول فقط زيادة في حجم الورم النسبي ولكنها اقل من الزيادة الحاصلة في مجموعتي السيطرة السالبة و الموجبة. أما وزن الجسم النسبي أظهر زيادة في المجموعة المعالجة بالفايروس عن طريق الحقن داخل كتلة الورم وانخفاض في المجموعة المعالجة داخل تجويف الخلب مقارنة مع وزن الجسم قبل بدء العلاج.

## **INTRODUCTION**

Levamisole hydrochloride ( a commonly used anthelmintic) is an Immunostimulating agent in animals and man(1). Its effects on humoral and cell-mediated immune response in several disease(2). Levamisole is non specific immunomodulator that is used in the treatment of Tuberculosis(3), AIDS(4), and Cancer(5).

Newcastle disease virus (NDV) is a member of the *Rubula* virus genus in the Paramyxoviridae family and is categorized in to three pathotypes depending on the severity of the disease that it causes in bird: Velogenic, Mesogenic, or Lentogenic (6). The oncolytic potential of NDV has been known for a long time(7), Subsequently, an attenuated NDV vaccine was found to cause significant regression of various human neoplasms including melanoma(8), and human colon carcinoma, larynx carcinoma, and pancreatic carcinoma(9). The purpose of the study was the treatment of mammary adenocarcinoma transplanted subcutaneously in mice with levamisole hydrochloride and LaSota strain of newcastel disease virus .

## **Material and Methods**

### **Newcastle Disease Virus (NDV).**

The lentogenic strain (LaSota strain) of NDV (LS. NDV) was obtained from Kendy company to veterinary vaccines production. The original stock was propagated in embryonated chicken

eggs (11days).After 96 hours from inoculation the virus was removed from the allantoic fluid by centrifugation for 30 min.4000rpm,4C°(10). NDV was quantified by the Hemagglutination and Hemagglutination inhibition tests(11) .Embryonated Infected Dose 50% (EID<sub>50</sub>) of virus was measured according to Karber method (12).

### **Tumor Cells and Transplantation.**

The tumor cells of murine mammary adenocarcinoma were obtained from live mice transplanted previously subcutaneously (S/C) (eight passage *invivo*) housed in Iraqi Center and Medical Cytogenetic transplantation of tumor cells S/C in mice was conducted according to (13).

### **Levamisole.**

Levamisole HCL,Bp98,Working stander,Assay- 99.74%, Reference stander ,Germany,Dr.Ehrenstarfer. Levamisole powder was dissolved in water and the mice was treated orally with dose (10mg/kg,B.W) through all time of experiment(14).

### **Relative tumor volume(R.T.V).**

R.T.V.was Determined according to phuangsab, *etal* (15).

$$\text{R.T.V.\%} = \frac{\text{Tumor Volume(day X)}}{\text{Tumor Volume(day 0)}} \times 100$$

### **Relative Body Weight(R.B.W).**

$$\text{R.B.W.\%} = \frac{\text{B.W in day x}}{\text{B.W in day 0}} \times 100$$

### **Laboratory animals.**

Fourty two female balb/c mice(22-26gm,10weeks)were obtained from Kendy Company ,housed in a controled environment and classified in to seven groups , Each of them contain six mice .Six groups were injected subcutaneously by (0.25ml) suspension of tumor cells.When the tumor nodul growth S\C About (9-12mm ),the animals were subjected to different treatments as follows.

**Group I :** The mice were treated with Levamisole orally through all time of experiment and injected with (0.1ml)from the LaSota strain of NDV (1024HU,EID<sub>50</sub>10<sup>9</sup>) intratumoral (I.T),six doses,three days intervals between doses.

**Group II :** Similar treated in the group I but injection of virus intra Peritoneally(I.P).

**Group III :** The mice were treated with Levamisole orally Only through all time of experiment.

**Group IV :** The mice were treated with Levamisole orally and Injected with ( 0.1ml) from allantoic fluid (fluid without virus) (I.T) six doses ,three days intervals between doses.Positive control group for group I.

**Group V :** Similar treated in the group IV but injected with allantoic fluid (I.P).(Positive control group for group II).

**Group VI :** The mice were injected with tumor cells only without treatment.( Negative control group for all groups).

**GroupVII :**The mice were left without any treatment or tumor.(Negative control group for body weight).

### **Statistical methods.**

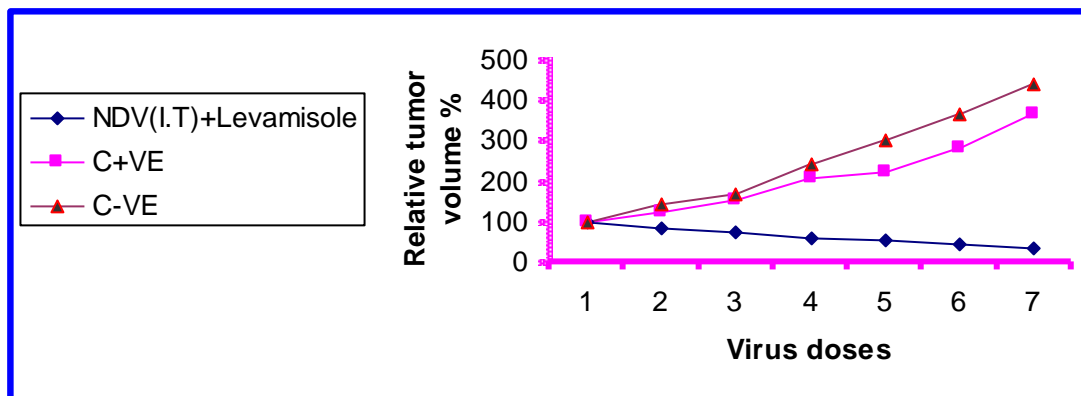
The differences in results were analysed using Least significant differences.

### **Results**

**The first group** , which was treated with levamisole orally and injected with LaSota strain of NDV (I.T) six doses, showed reduction of R.T.V after the first dose of virus

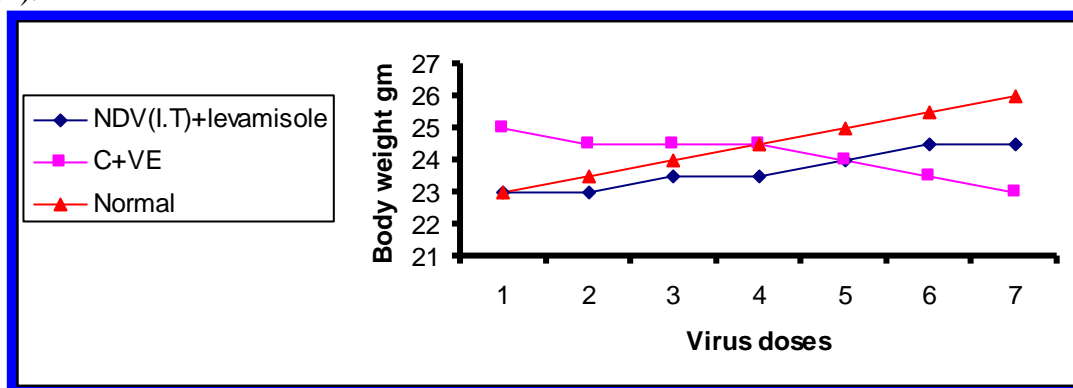
injection. This reduction was continuous to the end of experiment (18 days after treatment starting).

The percentage of the R.T.V. at the end of treatment was (33%) statistically significant ( $P < 0.0001$ ) compared with the tumor size before treatment starting, that means (67%) of tumor size was regressed.(Fig.1).



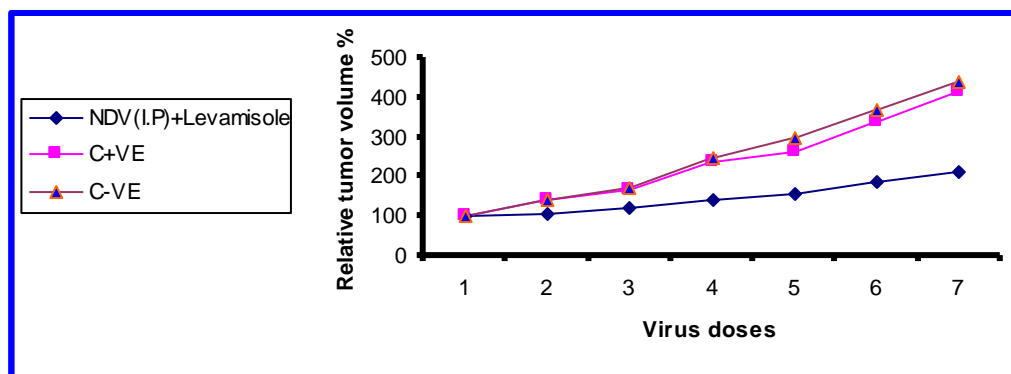
**Fig.1.Effects of levamisole and LS.NDV(I.T)on the R.T.V.**

The relative body weight in this group, showed an increase after the second dose of virus injection (6 days from the start of treatment) and the percentage of R.B.W. at the end of treatment was (6%) statistically no significant compared with the body weight before the treatment starting (Fig.2).



**Fig.2. Effects of treatment by levamisole orally and LS.NDV (I.T) on Body Weight.**

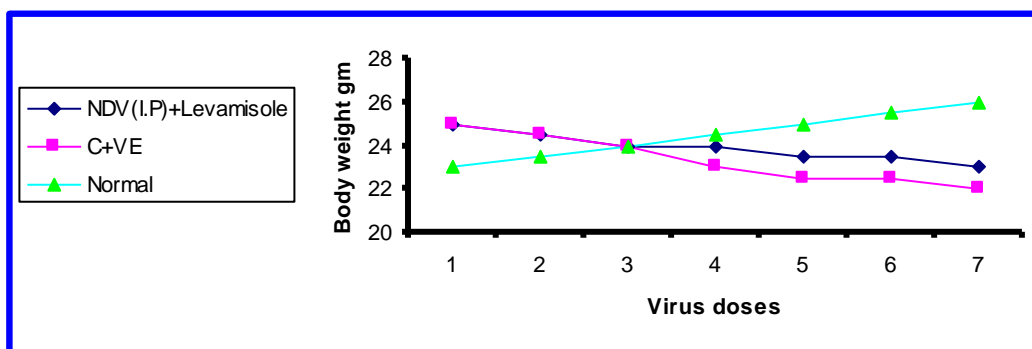
**The second group,** which was treated with levamisole orally and injected with LS.NDV(I.P) showed an increase in the R.T.V. after first injection of virus and this increased was continuous to the end of experiment. The percentage of R.T.V. (213%) statistically significant ( $P < 0.0001$ ) compared with tumor size before treatment, but less than the increase occurred in the negative and positive control groups. That means the tumor size in the treated group increased(1.13) times more than tumor size before the started of the treatment, while in the negative and positive control groups the increase was(3.39,3.12) respectively times more than the tumor size before the start of treatment(Fig.3)



**Fig.3.Effects of treatment by levamisole and LS.NDV(I.P) on The R.T.V.**

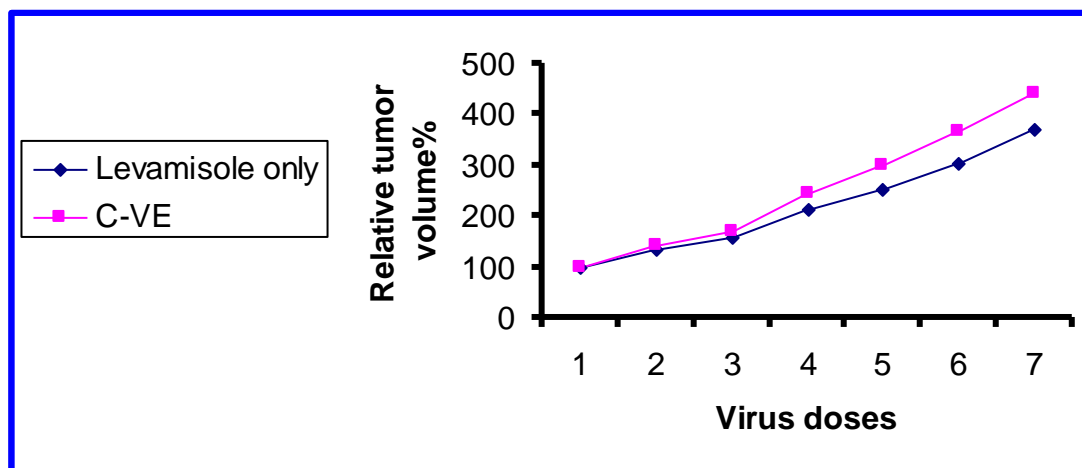
The Relative body weight in this group ,showed a decrease after first dose of treatment (three days from the start of treatment) and the decrease percentage of R.B.W. at the end of treatment was (8%) statistically no significant compared with the body weight before the start of treatment.

**Fig.4.**



**Fig.4. Effects of treatment by levamisole and LS.NDV(I.P) on the body weight.**

The third group, which was treated with levamisole only, showed an increase in the R.T.V. with a percentage of 371% at the end of experiment statistically significant ( $P < 0.0001$ ) compared with the tumor size before the start of treatment, but this increase was less than increase occurred in the negative control group. That means the tumor size increase in the treated group (2.71) times more than tumor size before treatment but in the negative control group (3.39) times more than tumor size before the start of treatment. Fig.5.



**Fig.5. Effect of Levamisole only on R.T.V.**

## **Discussion**

The first group which was treated with levamisole orally and multiple injection of LaSota strain of Newcastle disease virus (I.T) , showed a reduction in the relative tumor volume ,that means reduction in the tumor size compared with the tumor size before treatment and increase the Relative body weight after treatment. Parasad *et al* (16 ,17) used levamisole in combination with cimetidine, has been compared with Cimetidine alone in two trials, and was found to be effective at speeding regression of warts, with patients in the combination arm showing a mean regression time of 7-8 weeks compared with 11 weeks in the cimetidine alone group. In addition ,one non randomized controlled trial, Amer, *etal* (18) found Levamisole at 5 mg/kg for three days every two weeks lead to complete cure of warts in a group of 40 patients. Levamisole is non specific immunomodulator (19), can increase delayed hypersensitivity and augment macrophage chemotaxis and phagocytosis (20).

Baruah and Prasad(21) uses the levamisole as a growth facilitator in the culture of Scampi (*Macrobrachium rosenbergii* ) and they found significant differences in the growth parameters like percentage of weight gain ,percentage of specific growth rate and food conversion ratio among the control and levamisole treated animals besides the survival percentages of the animals treated with levamisole were better than the control.

NDV is an oncolytic viruses activated both innate and adaptive immunity. Innate immunity is a non-specific defence mechanism that is triggered immediately following pathogen detection and does not develop immunological memory for antigens .Adaptive immunity ,however, takes days to weeks to develop ,involves the generation of antibodies ,the activation,selection of immune cells,and immunological memory,making future immune responses more efficient (7). One strategy to develop oncolytic therapeutics is to select or design viruses that are especially sensitive to the antiviral properties of interferons. Viruses replication strongly suppressed in interferon –responsive normal tissues but still be able to flourish in interferon non responsive tumor cells (22) . Oncolytic viruses carry genes whose products are dedicated to short –circuiting the antiviral activity of interferons. So, tumor –selective oncolytic activity could be achieved by deleting or attenuating these anti-interferon gene products (23).

The reduction of tumor size through reduction in R.T.V. in the group treated with virus intratumoral (locally) more than in the group treated with virus intraperitoneally (systemically), that the virus injected (I.T) interact with specific cell receptors and endocytosis and replication of virus and killed the tumor cells directly (15) when injected the virus (I.P)

There are many barriers that prevent the virus from reaching the tumor and infecting cancer cells, such as, absorbed by liver, neutralizing antibodies, virion to access the tumor, it leave the circulation, leaking through the vascular endothelium against a gradient of interstitial fluid pressure. Additionally , infiltrating leukocytes limit cell-cell spread of the virus, either directly through antiviral activity or indirectly by the release of soluble inflammatory mediators (7).

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