Attenuated LaSota Virus Vaccine with a Urtica Pilulifera Extract In Treatment of Mammary adenocarcinoma in Mice

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Abstract

Background: The attenuated Newcastle Disease Virus (NDV) vaccine LaSota strain has been found to cause regression in volume of mammary adenocarcinoma transplanted subcutaneously in mice when injection locally (intratumoral) with Urtica pilulifera orally. The growth inhibition at the end of experiment was (77%) and statistically significant compared with the tumor volume in untreated group. While the growth inhibition in the group treated intraperitonealy (I.P) was (28%).The relative tumor volume was decreased in the group treated with virus (I.T) but increased in group treated (I.P) comparing with the tumor size at the begining of treatment. Histopathologically , there was an extensive area of necrosis and replacement by granulation tissue with mononuclear cells infiltration (lymphocytes, plasma cells and macrophages).

الخلاصة

وجد ان لقاح مرض فايروس نيوكاسل المضعف عترة لاسوتا يسبب تقهقر حجم سرطانة الغده اللبنية المغروسة تحت الجلد في الفئران عند حقنه داخل كتلة الورم مع تجريع الفئران بنبات القريص.حيث بلغت نسبة تثبيط حجم الورم عند نهاية التجربة(77%)مقارنة مع المجموعة غير المعالجة.بينما بلغت نسبة التثبيط في المجموعة المعالجة داخل تجويف الخلب(28%).كذلك سبب العلاج قلة حجم الورم النسبي في المجموعة المعالجة داخل كتلة الورم،بينما سبب زيادته في المجموعة المعالجة داخل تجويف الخلب مقارنة مع حجم الورم عند بداية العلاج. نسيجيا،ملاحظة منطقة تنخر شديد الذي يحل محله النسيج الحبيبي والخلايا الالتهابيه(اللمفاويه، والبلازميه وخلايا البلاعم الكبيره).

Introduction

There are different studies on the family Urticaceae showed a certain anti-tumor activity .The steroidal component of U.dioica roots extract reduced the tumor growth through inhibiting the membrane Na^+,K^+ ATpase activity in the prostate[1]or modulation of sex hormone biniding globulin to its receptor on human prostatic membranes in prostatic tumor patients [2] . Urticaceae is a safe therapeutic option for benign prostate hyperplasia syndrome [3]. Newcastel Disease Viruse is an avian paramyxo virus with a singlestrand RNA of negative polarity and coding for six genes (LP, HN, FP, NP, Pp and MP) [4] .The tumor cells infected with NDV can be killed directly by the virus [5] or indirectly through an immune system response to the infection [6]. The immune system uses a variety of approaches to kill virus-infected cells, including attack by cytotoxic cells [7] and attack by anti-virus antibodies [8].

Material and methods

Tumor cells and transplantation

The tumor cells were obtained from live mice housed in Iraqi center of cancer and medical cytogenetics.It was transplanted previously the S/C with murine mammary adenocarcinoma (seven passages *invivo*) (fig.1). After sterilization of the outside of tumor

nodule, the tumor cells were aspirated by needle gage (18mm), suspended in phosphate buffer saline (PBS), centrifugation (1000rpm, 10min., 20c^{o}), the supernatant was left and the sediment was resuspended in PBS and transplantation of tumor cells was done S/C in mice[9].

Tumor volume.

Tumor volume (T.V)=A.B^2\ 2 according to Grote,*etal*(10).A=length. B=wide.

Growth inhibition(G.I). According to Phuangsab, *et al*(11).

$$G.I.\ \% = \frac{T.V.\text{in untreated group} - T.V.\text{in treated group}}{T.V.\text{in untreated group}} \times 100$$

Relative tumor volume (R.T.V.). According to Phuangsab, *et al* (11).

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

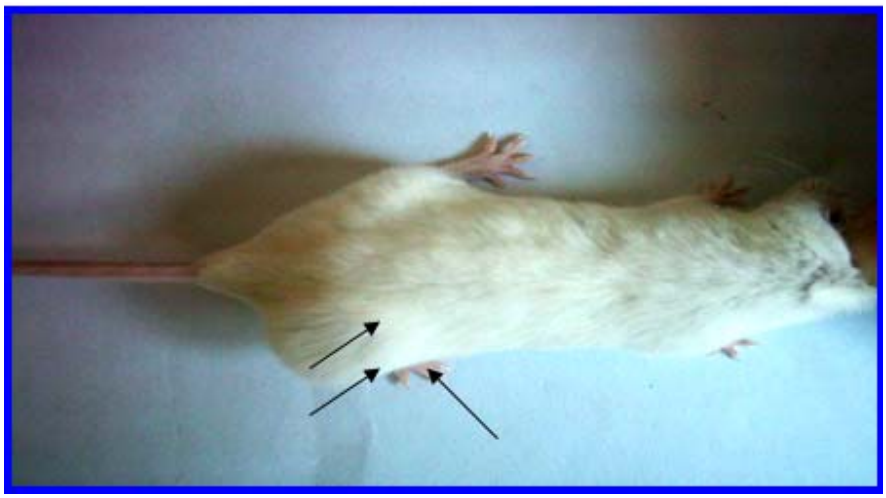

Fig.(1) .Mice transplanted S/C with seven passages of mammary adenocarcinoma cells.

Newcastle Disease Virus (NDV).

Attenuated NDV (LaSota strain) was a gift from Kendy Company for veterinary vaccines production .Amplification of the original stock was done by inoculation through (10day) old chick emberyos .Four days after inoculation ,virus was removed the allantoic fluid by centrifugation for 30min .,4000 rpm ,4c^{o} [12] and the measurement of emberyonated infected dose (EID_{50}) and hemagglutination unit (HU) were done according to Karber method[13].

Medical plant(Urtica pilulifera).

The plant Urtica pilulifera was obtained from Iraqi center of cancer and medical cytogenetics .The preparation of its extract was done according to Sakai *et al*(14).

Laboratory animals.

Thirty female balb\c mice(22gm,10weeks)were obtained from kendy company ,housed in a control environment and classified in to six groups each of them contained five mice. All mice were injected subcutaneously by (0.25ml) suspension of tumor cells.When the S/C tumor nodul growth about (8-12 mm). The animals were subjected to different reached treatments as follows.

Group I: The mice were treated with(1ml) U.pilulifera extract orally four

doses and injection(0.1ml)from the LaSota strain of NDV (1024 HU,$EID_{50}10^9$) intratumoral (I.T),four doses,three days intervals between doses.

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

Group III : The mice were treated with (1ml) U.pilulifera extract orally only four doses, three days intervals between doses.

Group IV : The mice were treated with (1ml) U.pilulifera orally and injection(0.1ml) from allantoic fluid(fluid without virus) (I.T) four doses, three days intervals between doses.

Group V: Similar treatment in the group IV but injection of allantoic fluid (I.P).

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

Histological examination

At the termination, all animals were autopsied and the tumor mass was taken for histological examination. The tissues were fixed in buffer (10%)formalin, sections were routinely prepared and stained with eosin and hematoxylin (H&E)for microscopic examination[15].

Statistical methods

The differences in results analyzed by ASA (2001) program and uses Least Significant Differences test (LSD)

Results

Growth inhibition of tumor

The growth inhibition of tumor volume had been increased after three days from the start of treatment and this inhibition was continuously increased till the end of experiment after four doses administration of virus and plant compared with negative control group (Table 1) . The inhibition of tumor volume in the first group was statistically significant (P<0.01) compared with the second group and (P<0.001) compared with third group. (fig.2).

Table 1.The growth inhibition compared with negative control Group.

Groups	Day 3	Day 6	Day 9	Day 12	significant
Group I	27%	48%	70%	77%	P<0.0001
Group II	3%	19%	12%	28%	P<0.01
GroupIII	6%	7%	13%	15%	P<0.05
Group IV	3%	10%	13%	4%	No significant
Group V	1%	4%	4%	2%	No significant

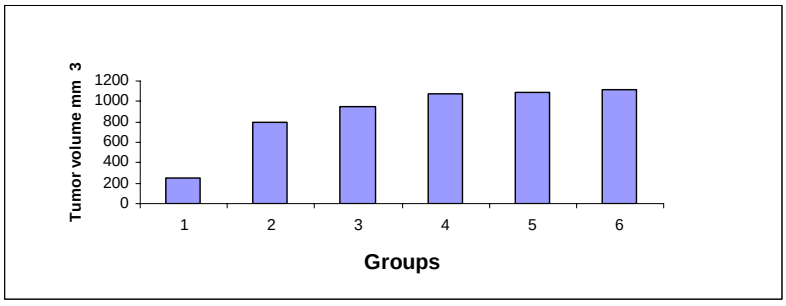

Fig 2 .The effect of treatment on groups at the end of experiment**.**

Relative tumor volume(R.T.V).

The R.T.V was reduced in the first group after treatment ,while increased in the others compared with tumor size at the start of the treatment. The increment in R.T.V in treated groups (second and third groups) was less than that occured in others groups (Table 2) .The

regression of tumor size in the first group was statistically significant (P<0.0001) compared with the second and third groups at the end of experiment (fig 3).

Table 2. The effect of treatment on R.T.V. in groups through days of experiment.

Groups	Day 0	Day 3	Day 6	Day 9	Day 12
I	100%	89%	80 %	53%	44 %
II	100%	111%	116%	125%	133 %
III	100%	122 %	154 %	169%	179 %
IV	100%	121%	136%	156%	189%
V	100%	130 %	159%	188 %	208%
VI	100%	127 %	161 %	190%	210 %

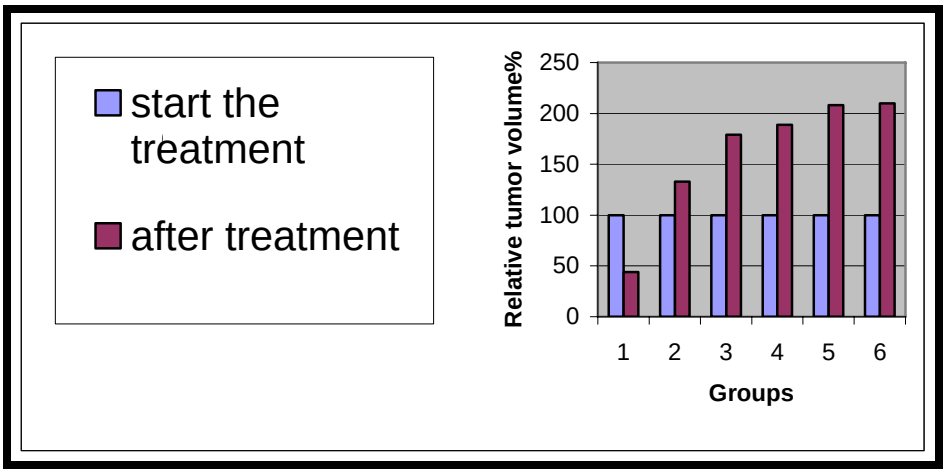

Fig .3. The effect of treatment on R.T.V in groups at the end of experiment.

Macroscopic and Histological appearance.

The macroscopic effects of treating tumors in the first group show reduce the tumor size without any damage to the overlying skin. In contrast with the second mouse treatment of tumor of identical size with allantoic fluid was followed by rapid tumor growth (fig.4).

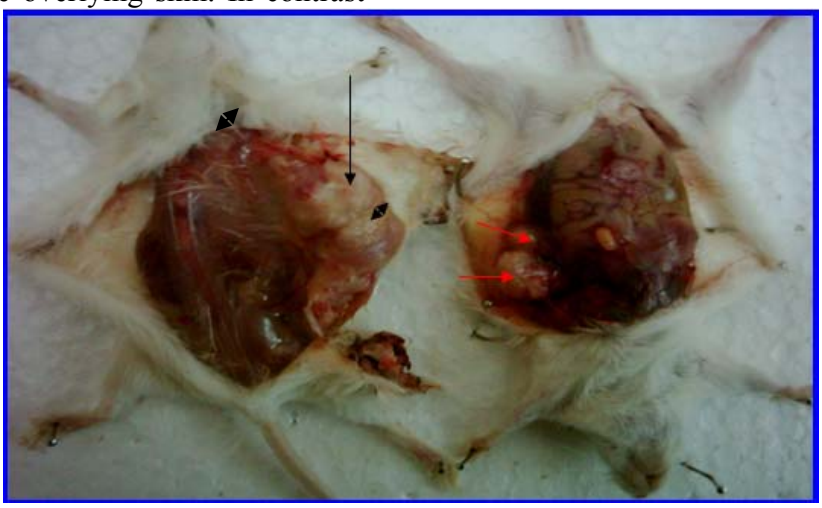

Fig.4.Shows reduce of tumor size in treated group with NDV(I.T) → compared with treated by allantoic fluid(⟶).

Histological sections of tumors were obtained 12 days after treatment in the group treated with NDV intratumoral ,the treatment led to a total replacement of all viable tumor cells by granulation tissue and sever mononuclear inflammatory cells infiltration such as lymphocytes, plasma cells and macrophage .The tumor cells were necrosis and vacculation of cytoplasm in others (lesion like a apoptosis) (fig.5).Histological sections of allantoic fluid treated mammary adenocarcinoma tumor showed viable tumor cells with identical characteristics to those were seen previously [16].

Fig.6.shows the histological effects of treating tumor with U.pilulifera and injection of NDV intraperitoneally, necrosis in some areas of tumor section and mild to moderate mononuclear inflammatory cells infiltration such as lymphocytes ,plasma cells and macrophages and shows area of tumor cells without necrosis.

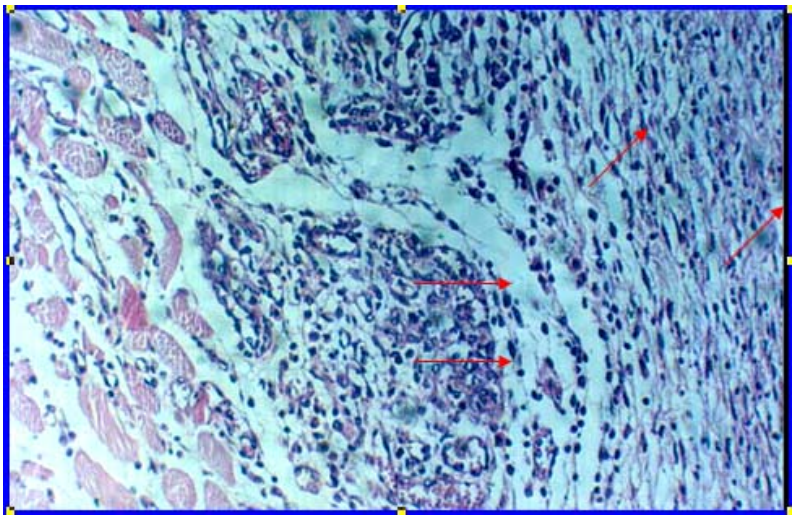

Fig.5.Massive area of necrosis and vacculation of cytoplasm tumor cells with inflammatory cells (H&E)(200X).

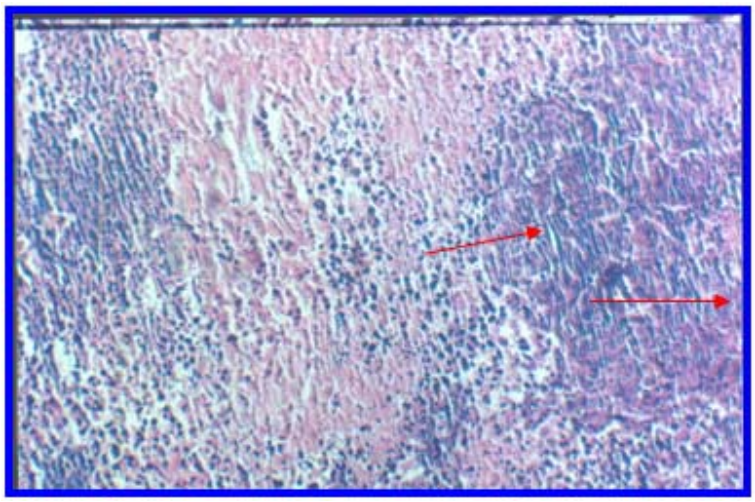

Fig.6.Area of necrosis with inflammatory cells and shows the area of tumor cells without necrosis (H&E)(200X).

Discussion

Much attention has been given to the primary prevention of cancer in daily life. Suppression of the tumor promotion step by the functional constituents of plant resources is expected to prevent cancer development. A number of products has been studied for anticancer activity on various models .This has

resulted in the availability of more than 3000 effective anticancer drugs [17] .

In this study,the group treated by LaSota virus(I.T)with U.pilulifera orally showed an increasing in growth inhibition and a decrement in relative tumor volume which means reduction in tumor size . The U.pililufera contained isoflavone genstein glycoside leads to production of lipopolysaccharide induced tumor necrosis factor-alpha, interleukin-1 and IL-6 in from both the liver and serum [18]. The extract contains two caffeicacid derivatives ,3,5 dicaffeoyl quinic ester and dicaffeoylmalic ester; they significantly inhibit the cell growth and synthesis of RNA,DNA and protein in human colon adenocarcinoma cells [19].The effect of U.pilulifera herb extraction on growth of carcinoma may be due to the presence of bioactive compounds such as polysaccharide [20] that reduces tumor cell proliferation by decreasing sialic acid amount and phospholipids in cancer cell membrane[21].

In addition, the attenuated NDV (LaSota strain) exerts oncolytic effects and it is found to be able to kill a wide rang of transformed cells by apoptosis through activation of caspase-3 [7].The oncolytic virus was developed based on its ability to only replicate in cells that lack cytochrome P_{53}-acommen feature of cancer cells leading to lack the EIB-55K gene product ,which was normally required for degrading the cellular P_{53} protein during viral infections allowing to only replicate in and destroy cells that lack P_{53},such as tumor cells[22].In some patients with solid tumors who were treated with NDV under went a complete response, and several patients experienced partial responses,in these patients tumor growth was stabilized for months to years,and in one case histological evidence of tumor regression was accompanied by immune infiltration[23].

The inhibition of tumor size and histological changes(necrosis and inflammatory cells) in the groups treated with LaSota strain virus intraperitonealy less than that occurred in the group treated intratumoral because the virus was little or no spread from the primary site of injection, that means intratumoral administration was probably not effective against disseminated disease[24].In addition,when injection virus (I.P) ,the body contains neutralizing anti-virus antibody lead to decrease the amount of virus reach the tumor site[25].

In conclusion, this study has shown that the NDV is preferentially killed tumor cells and activated the immune response.NDV may therefore be recommended for anti-tumor activity.

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