Anti-tumor Activity Of Virulent Newcastle Virus With Urtica pilulifera On Mammary Adenocarcinoma In mice

Abstract:
The anti-tumor activity of the Virulent Newcastle Virus (VNV) \( (LD_{50}10^9) \) and water extract of Uritica pilulifera leaves (U.P) were evaluated against the murine mammary adenocarcinoma transplanted subcutaneously in mice. The activity was assessed using growth inhibition of tumor volume, relative tumor volume and histopathological examination. Intratumoral injection of (VNV) and Uritica pilulifera crude extract was given different doses resulted in a prominent suppression of tumor statistically orally with significant with 82%, the relative tumor volume was also reduced and the suppression of tumor size in this group more than others. Histopathological examination show massive necrosis associated with fibrosis and lymphocytic with macrophagic infiltration.

Introduction
The idea of using viruses in tumor therapy is based on observation of remission of human cancers following natural viral infections such as mumps or measles(1). Newcastle Disease Virus(NDV) has been suggested as a very promising agent for tumor therapy(2) because this avian virus in mammalian species including man has interesting anti-neoplastic (3) as well as immune stimulation(4) properties, it can oncolytic (5) and it can activate host immune cells to produce cytokines (6) and to become cytotoxic against tumor cells(7). Herbal remedies are widely used for the treatment and prevention of various diseases and often contain highly active pharmacological compounds (8). Uricaceae was reported as one of the effective medical plants, these plants are being consumed without any report of adverse effects (9) The U. pilulifera L. is commonly used as a remedy for diabetes mellitus (10), anti-tumor activity (11), and anti oxidant effect(12). According to the previous studies on the NDV and plant extract, it was of interest to estimate its anti-cancer effect on mammary adenocarcinoma transplanted in mice.

Material And Methods
Newcastle Disease Virus(NDV).
The virulent lytic strain NDV (Iraqi strain) was obtained from department of Pathology and Poultry Disease, College of Vet. Med. Baghdad University. A stock of infectious virus was propagated in embryonated chicken eggs (9-11days) harvested from the allantoic fluid, purified from debris by cold centrifugation (3000 rpm ,30 min ,\( 4^\circ C \))(13).
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NDV was quantified by the hemagglutination and hemagglutination inhibition test (14). The measurement of Emberyonated Leathal Dose 50% (ELD_{50}) of Virus was coducted according to the Karber method(15).

Plant(U.pilulifera)
The leaves of U. p were obtained from Iraqi Center of Cancer and Medical Cytogenetics.

Extraction and separation
   Twenty-five grams gram of U. p powder were exhaustively extracted with 300 ml of Distille water, the mixture was put in water bath (50°C,24 hours), filtered, centrifuged at (4000 rpm ,30min) and the extract concentrated by rotary evaporator(16).

Tumor cells
   The cell line of murine mammary adenocarcinoma (AM3) were obtained from Iraqi Center of Cancer and Medical Cytogenetics. Transplantation of tumor cells subcutaneously according to(3).

Laboratory animals.
   Female balb \C mice (22-26gm) (8-12 weeks) were procured from Iraqi Center of Cancer and Medical Cytogenetics in March 2004. They were housed in boxes in a control environment (temp.25°C) with standard laboratory diet and water. The animals were classified in to six groups each of them contain five mice. All of them were injected subcutaneously by (0.25)ml suspension of tumor cells. When the tumor nodule growth S\C and reached about (6-12 mm) all animals were subjected to different treatments as follows.

Group I : The mice were treated orally with (1ml) U.pilulifera four doses and injection of the NDV (0.1ml,LD_{50}10^{9}) directly in the tumor (Intratumoral) (I.T) four doses, three days intervals between doses.

Group II : Similarly treated as in the group I, but injection of the virus was through peritoneum (Intraperitonealy).

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

Group IV : The mice were treated with (1ml) U.P orally and injection of allantoic fluid (virus- free) directly in the tumor(I.T). four doses, three days intervals between doses. This group is considered a positive control group for group I.(C+VE).

Group V : Similarly treated as in the group IV but injection of allantoic fluid was done Intraperitonealy .Positive control group for groupII.

Group VI: The mice injected with tumor cells only without treatment. A negative control group to all groups(C-VE).

Determination of tumor volume(T.V).
The tumor volume of treated mice and tumor bearing mice was estimated according to Grote .etal (17). Tumor volume=A.B^{2}/2= mm^{3}
\[ \text{A=Length} \quad \text{B=Wide} \]

Determination of Growth inhibition(G.I).
The measurement of G.I% was conducted according to Phuang sab, etal (18).
T.V in untreated group –T.V in treated group
G.I%=(T.V in untreated group –T.V in treated group) x100

**Determination of Relative Tumor Volume (R.T.V)**
The determination of R.T.V. was followed according to Phuangsab etal(18).

\[ \text{R.T.V} \% = \left( \frac{\text{T.V(day X)}}{\text{T.V(day 0)}} \right) \times 100 \]

**Pathological study.**
Postmortem examination was done on dead mice. Tissue samples were taken from tumor mass and subjected to routine histopathological study, consisted of fixation and dehydration. Five to six microns-thick sections were stained with haematoxylin & eosine staining and examined with light microscope at various magnifications(19).

**Statistical analysis:**
Program of SAS(2001) to analysis the result of study by Least Significant Differences(LDS) was used in this study.

**Results**

**Tumor volume.**
The first group which was treated with U.pilulifera orally and injected with virulence newcastle virus directly in the tumor(I.T), resulted inhibition of tumor growth after three days from first dose. This inhibition of tumor growth continued after four doses (12) days from start of treatment. The percent of growth inhibition at the end of treatment was (82%), statistically significant (P<0.0001) compared with negative group control, and (78%) statistically significant (P<0.0001) compared with positive group control.(Fig.1).

The second group which was injected by the virus intraperitonealy, showed an inhibition of tumor growth after first dose injected and the percent of growth inhibition at the end of treatment was (22%), statistically significant (P<0.01) compared with the negative control group, and (20%) compared with the positive control group.(Fig.2). The third group which was treated with U.pilulifera orally only, showed inhibition of tumor growth and the percent of growth inhibition at the end of treatment was (14%) compared with the negative control group (Fig.3). The growth inhibition in the first group was more than statistically significant (P<0.0001) compared with second & third groups.
Fig(1). The inhibition of tumor growth after treatment by multiple injection of NDV(I.T) with U.P (orally).

Fig(2). Anti-tumor activity of NDV(I.P) with U.P (orally).
Relative tumor volume(R.T.V)

The first group, show that reduction in (R.T.V) was observed after three days from the start of the treatment, this reduction were continuous to end of the experiment(four doses,12 days)from the start of the treatment. The percent of R.T.V was (35%) statistically significant(P<0.0001) compared with the tumor size before the start of the treatment(fig.4). That means (65%) of tumor size was regression. The second group which was injected by virus intraperitonealy show an increase in R.T.V about(45%) at the end of experiment compared with the tumor size before the start of the treatment, but this increase was less than the increase which occurs in the negative (117%) and positive (113%) control groups compared with tumor size before the start of the treatment(fig.5), that means the tumor size in the treated group was less than about(72%) from the tumor size in untreated groups at the end of experiment.

The third group which was treated with Urtica pilulifera only show an increase in the R.T.V (77%) at the end of experiment compared with the size of tumor at the start of experiment, but the tumor size in this group was less than about (40%) from the tumor size in untreated group at the end of experiment(fig.6).
Fig(4). Effect of treatment by NDV(I.T) and U.P on R.T.V.

Fig(5). Effect of treatment by NDV(I.P) with U.P on R.T.V.
Histopathological examination.

The histopathological effects of treating tumors with intratumoral injection of virulence NDV with U.pilulifera (orally), show wide area of massive necrosis of tumor cells associated with infiltration of inflammatory cells such as neutrophils, plasma cells, lymphocytes and macrophages, in addition fibrous connective tissue replacement the necrotic area with vacuolation in cytoplasm of tumor cells like apoptosis (fig. 7). The histopathological effects of intraperitoneally treatment by virulence NDV with U.pilulifera (orally), necrosis in some area of tumor associated with infiltration of lymphocytes and macrophages was observed. There are present area of tumor cells without necrosis (fig. 8).

Fig(6). Effect of U.P only on R.T.V.

Fig(7). Massive area of necrosis with inflammatory cells and fibrous connective tissue. (H&E 200 X)
Discussion

In this study, virulence NDV (Iraqi strain) had a pronounced anti-tumor effect when it was given by both local (intratumoral) and systemic (intraperitoneal) routes. Virulent strain was selected for the study since it has been previously given to human and animals by a variety of routes and had demonstrated a good safety profile at the dose used (3, 20). A dose of $1 \times 10^9$ ELD$_{50}$ was administered I.T in the first group lead to inhibition of tumor growth and relative tumor volume. Phuangsb et al. (18) administered NDV viral oncolysate preparation to twelve mice with subcutaneous xenografts of epidermoid carcinoma were intratumoral treatment, the virus caused an average of (81%) growth inhibition and reduction in relative tumor volumes for the mice treated with NDV were also significantly.

Cytolysis strains of NDV were selectively replicate in and rapidly kill adverse group of human tumor cells (21). Oncogenic transformation increase the sensitivity of malignant tumor cells to NDV cytolysis (22). Stodji et al. (23) suggested that this selectivity is based upon a cancer-specific down regulation of the interferone pathway within the tumor cells that leaves these cells open to infection with certain types of lytic viruses.

The inhibition of tumor size and the histopathological changes (necrosis and inflammatory cells) in the group treated with NDV (I.T) were occured more than in that group treated with NDV (I.P), there are numerous possibilities explain the mechanism of this such as a- binding of virus to normal tissues or cells, b- inactivation of virus before it reaches the tumor site or c- regrowth of virus resistant tumor cells after the initial challenge (18).

In addition, Urtica pilulifera herb extract showed agood antioxidant activity, this support the anti-cancer effect of the plant extract (11). Also, Seeram et al. (24) found that the flavonoids, especially these contain C-7 and C-4 hydroxyl groups act as anti-mutagen and anti-malignant agent and the phytochemical studies showed that U.P contains the same flavonoid (25). Mak et al. (26) explained the mode of action of genistein glycosides as anti-tumor drug. U.pilulifera contains isoflavone genistein glycoside and tyrosine kinase which inhibits proliferation of prostate cancer cell through the induction of apoptosis (27).

The increase of inflammatory cells (lymphocytes and macrophages) in necrotic area of tumor is due to the fact that these cells are the first one to arrive at sites of infection, where they can...
release chemokines and proteases that can in turn recruit both specific and nonspecific immune effector cells (28). They can also release toxic granules against neighboring cells, suggesting their potential anti-tumor activity (29).

References

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