

ANTI-CANCER ACTIVITY OF NIGELLA SATIVA

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ABSDRACE: An extract of Smilax china, Hemidesmus indicus and Nigella Sativa on the ratio 3:2:1, prepared by boiling in water and concentrated could completely cure cases of oral cancer diagnosed by modern methods. Cytotoxic studies with the three components showed activity in Nigella sativa at a concentration of 25 microgram equivalent of the dry powder against Dalton's lymphoma ascites cells. Animal experiments indicated the retarded growth of ascites as compared to the controls with a longevity of 90%.

Introduction

The system of ayurvedic medicine consists of plant products as well as chemicals and in several cases extracts of plants are mixed together as a drug of choice rather than the individual extracts as such due to the interaction of various components present in them. Many plants have been tested for biological activities including anticancer activity by systematic procedure and a few compounds like Vincristine have proved successful as an anticancer drug. We have come across a preparation (given by Rajagopalan, K.) used as a medicine for treatment of wounds which are naturally not completely cured by wound healing drugs. This remarkably property may be due to the possibility of an ingredient which may act as anticancer drug, and thereby

completely healing the wound which have been of cancerous origin.

It was therefore decided to investigate the extract for the possible presence of anticancer property by in vivo and in vitro cytotoxic studies. The response of this medicine on oral cancer patients are also presented.

Materials and Methods

The investigation consists of experiments to study the cytotoxic effect of an Dalton's lymphoma ascites cell grown in Swiss albino mice.

Preparation of the extract

The preparation consists of Smilax China and Hemidesmus indicus. Nigella sativa in the ratio 3:2:1. S. China and H. indicus were cut into

small pieces and *Nigella Sativa* added after powdering. They are mixed together and boiled with water concentrated and filtered to have a concentration of 30 mg, 20 mg and 10 mg of the drug respectively/per ml of the extract. The drug was sweetened by sucrose. The syrupy solution is found to be stable when stored at 4°C in the refrigerator.

In vitro cytotoxic studies

Dalton's lymphoma cells were grown in the Swiss albino mice in ascites form by transplanting the cells from other tumour bearing mice. Ascites develop within 10 to 14 days and the cells are drawn carefully with a syringe under sterile conditions. These are washed with a phosphate buffer containing saline (0.2 M phosphate buffer pH 7.4 containing 0.15 M sodium chloride), several times to remove them from RBC and other cells. These are counted and diluted with PBS. A serial dilution of the extracts were mixed with cells to have a concentration of 1 million cells per ml and incubated at 37°C for 3 hours. Immediately after the incubation 1% trypan blue solution was added to the medium and in this process the dead cells absorb the dye and could be easily counted under the microscope. The cytotoxicity could be measured by the counting the number of dead cells for different concentrations of drug and the minimum concentration of the drug required for 100% cytotoxicity determined. The cytotoxic effect of the combined extract as well as the individual extracts were determined in the alcohol and water extracts.

Animals experiments

In vivo cytotoxic studies were carried out on Swiss albino mice. Ascitic fluid was drawn from the peritoneal cavity of the tumour bearing

albino mice and washed with PBS to free them from cell debris. RBC etc. And diluted in PBS at a concentration of 1 million cells per ml. This cell suspension was transferred to the peritoneal cavity of the 16 albino mice at a concentration of one million cells. The animals were divided into two groups of eight each, one million cells. The animals were divided into two groups of eight each, one experimental and the control. All the experimental animals were given 1 ml of the extract by oral feeding from the second day after transplanting the tumour and continued for twenty days. The control animals were left as such without any medicine. The development of ascites was examined along with the longevity of the animals in both experimental and control. This experiment can give an idea regarding the role of the medicine in preventing the multiplication of the cells in the peritoneal cavity and thereby the possible effect in cancer treatment.

Case Report

We have been able to use this medicine for the treatment of oral cancer and the details of two patients are presented here.

An 84 year old female patient having complaint of ulcer on the left cheek with 2"x1" size was brought to our hospital on 24-1-1987, after having it diagnosed as Ca. cheek at Amala Cancer Hospital Oncology Department vide their H.No. 919. The patient was examined and noticed pus discharge from left cheek inside, swelling externally (left cheek) and severe pain. The patient was admitted on the same day and the following medicines were administered for seven days.

- 1) Gulgulu tikta quatham 15 ml-B.D.
Kanchanara gulgulu gulika 1 B.D. with quatham.

- 2) Rasasindooram 500 mg-B.D. with honey.
- 3) Triphala churnam for gargling.
- 4) Pinda thailam for external application.

Having found no response for seven days we had administered our medicine in the form of water extract in the ratio S. China, H. indicus and Nigella Sativa (3:2:1). The extract was prepared as a decoction by boiling in water and concentrated. A positive response was noted on the 7th day of the administration of medicine. The pain began to reduce, swelling subside and pus discharge also reduced. After one month treatment 75% of the ulcer was healed, no pain or discharge. The patient was discharged on 16-3-1988 with advice to continue the medicine. The medicine was continued for one more month and at present the ulcer is completely cured and there is only a small scar.

An old lady of 80 came to our hospital on 31-3-1986 with a complaint of ulcer on Rt. Cheek of two years duration. On examination this ulcer was 2x1½ inch size and severe pain and pus discharge was noticed. This case was diagnosed as cancer cheek by Amala Cancer Hospital via H.No. 4917. The patient was treated as an outpatient and the following medicines were prescribed for 30 days. On 30-8-1986 the patient came to our OP having no relief. Hence she was given the new medicines and on 23-9-1986 she came our OP having 50% of ulcer healed, pus discharge has reduced. The patient continued the treatment for two months. The ulcer is completely healed and even today she is healthy.

Results and Discussion

The cytotoxic effect by in vitro study is summarized in table I. which indicate that the water extract as well as the alcohol extract

were active against Dalton's lymphoma cells but better activity is obtained in the case of an alcoholic extract. It is observed that S China and H. indicus did not possess any cytotoxic activity when this water or alcoholic extracts were tested while Nigella Sativa extract showed remarkable activity. This suggests presence of an active ingredient in the seeds of Nigella Sativa and is soluble in alcohol indicating its lipid nature which was also confirmed by oily appearance when spotted on filter paper. The unsaturated nature of this preparation was also detected by the production of iodine colour when exposed to iodine vapour. Further studies with the alcoholic extract indicated 100% cytotoxicity for concentration of 25 microgram equivalent of this original powder.

In the animal experiment the group which was given the drug by oral feeding showed a remarkable reduction in the growth of ascites as compared to the controls. The control animals developed ascites within sixteen to twenty days and lived upto 25 to 30 days as against the experimental animals which lived for more than fifty days. The general nature of the animals were better in the case of experimental animals.

Table I

Solvent used	Concentration of the drug	Samples used			
		Nigella sativa	H.indicus	S. China	Combined
Water	5mg	100	-	-	-
	0.5mg	100	-	-	-
	0.05mg	0	-	-	-
	0.25mg	0	-	-	-
Alcohol 95%	5mg	100	-	-	-
	0.5mg	100	-	-	-
	0.05mg	100	-	-	-
	0.025mg	100	-	-	-

The drug extract in water or alcohol treated with 1×10^6 million cells of Danlton's lymphoma ascites and dead cells are counted after three hours at 37°C , by trypan blue exclusive method.

Table –II Ascites tumour development and survival of animals

	Average wt. of ascites after 20 days gms	Average longevity	Increased life span T-Cx100 C
Test T	8.3	51	-
Control C	152	27	90%

Even though cytotoxic principle was detected only in *Nigella sativum* the other ingredients, also might have some important role. The active ingredients are soluble only in alcohol and therefore the effective transportation of the material is a problem unless some physical changes take place as in the formation of liposomal type combinations and we believe that this type of a change can take place in the presence of other two ingredients. Further the absorption of lipid type material also require the formation of lipoprotein and their transportation to the cancerous tissue for better action, and in the presence of the other two ingredients this may become easier thereby the drug becoming more effective. The cytotoxic effect of the *Nigella Sativa* is far better as compared other cytotoxic agents since it gives 100% cytotoxicity for a concentration equivalent to 25 microgram against 200 microgram for *ixora coccinea*, *ixora javanica* turmeric. (1,2,3).

It is found that a treatment by this medicine could cure the symptoms in carcinoma cheek in

the case of two patients after proper diagnosis by modern methods. The two patients are healthy after the treatment and they are alive. The active ingredient responsible for the cure must be the seeds of *Nigella Sativa* which had been found to contain cytotoxic principles. The animal experiments also revealed the potential anticancer nature of the substances as evidenced by the reduction in the tumour growth. The active principle is a fat soluble substance, and the others ingredients are probably involved in the absorption and transport to the cancerous cell. We can assume that this cure is due to the medicines, as we could not get any response with the usual medicines administered.

Further work in the purification and identification of this active ingredients are in progress. In subsequent animal experiments we have found that 30 mg equivalent of the drug powder of *Nigella sativum* was lethal to Swiss albino mice when administered intraperitoneally. This shows that the other two components may have a nullifying effect on the possible toxic materials of *nigella sativa*. Therefore there is a possibility that the extract given in combination had a better effect than the separate administration of the individual extract.

References

1. K.R. Panikkar; P.Bhanumathy; & P.N Raghunath; Amala Research Bulletin. 5, 39-41 (1985).
2. V.Vijayakumari,; Salomi John and K.R. Panikkar: Amala Research Bulletin.6, 18-19 (1987).
3. Ramadasan Kuttan; P. Bhanumathy; K. Nirmala & M.C George : Cancer Letters. 29, 197-202 (1985).

