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INFLUENCE OF SOME HIGHLIGHTED VEGETABLES ON CANCER PREVENTION IN DAL BEARING MICE

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Abstract

The present study is an attempt to understand the role of some highlighted vegetables like Tomato, Carrot, Onion, Cabbage, Soy bean, and Chick pea with Vitamin D_3 when given together in vivo as chemopreventive agent against experimentally DAL (Dalton's ascites lymphoma cell line) induced mice carcinogenesis.

The anticarcinogenic potential of this micro-nutrient rich food has been assessed by their ability to modulate certain life style in the early stages of DAL induced liver carcinogenesis in mice. Survival study of all experimental mice has a 62.5% which fed by above vegetables to cancer induced mice whereas the group fed without vegetables are showed 28.5% survival during 30 days of study period. The preliminary enzyme like SGPT and SGOT level change also observed in those mice.

Introduction

Dietary nutrient rich foods Tomato, Carrot, Onion, Cabbage, Soy bean, and Chick pea are employed for the treatment of various types of cancers. Research team led by Rutger et.al., has revealed that widely consumed cruciferous vegetables have shown to inhibit cancer in rodents induced by carcinogens. The National institute recommends eating five serving of fruits and vegetables each day could be having cancer

prevention property. The American cancer society estimates that more than two third of cancer can be prevented through life style modification and diet alone.

Malignant cells show several biochemical differences when compare to normal cells, The anti-tumour property of dietary nutrient is well established and is therapeutically used in human leukemia and other forms of malignancies as a cytotoxic agent.

MATERIALS AND METHODS

Vitamin D_3 capsules were obtained from Glaxo Smithkline Pharmaceutical Limited, Mumbai. DAL - Cell line were obtained from AMLA Cancer Institute, Trissur, Kerala. All other chemicals and reagents used were of analytical grade and vegetables used were garden fresh. The *in vivo* studies of this work are carried out with the permission of animal ethical committee "Committee for the purpose of control and supervision of experimental animals" (CPCSEA Registered Number 0367/01/C/CPCSEA India) for the care and use of lab animals.

Animal and Diet:

Male mice are obtained from Amla Cancer Research Centre Trissur, Kerala, weighing 16-22 gms at beginning of the experiment were used. The animals were kept in wire mesh cages (4-6 rats/cage) at constant temperature at 23 ± 1°C, relative humidity 50-60% with a controlled light and dark rhythm provided with semi purified basal diet and water. Mice were acclimatized for 1 week before the commencement of the experiment. We followed the recommendations of the NIH guide for the care and use of laboratory animals for the maintenance, treatment and sacrifice of the animals used in this study. The same pattern was followed for all type of studies in our work.

Experimental design:

The mice was divided into 4 groups as group A, B, C and D, each group contain 8 animals. Group A, B and C mice were DAL induced. Among those, the group A received dietary nutrients rich food like

tomato, carrot, onion, cabbage, soy bean, and chick pea as regular food along with vitamin D_3. Group B mice received vitamin D_3 and basal food. Group C mice were treated as DAL control and group D mice served as normal control.

Cancer was initiated by single intraperitoneal (i.p) injection of DAL at the dose of 2ml/kg body weight in 0.9% Nacl solution (buffer solution) to A, B and C groups after 30 days of the treatment.

The experiment was continued for 30 more days and animals were maintained with the basal diet. Animals in different groups were weighed individually at one week intervals and weights are recorded. A single Elico balance is used through out the experiment for weighing all the animals.

All experimental groups were sacrificed by proper ether anesthesia. The blood sample of 1-2 ml from each animal was collected from retro orbital vein with the help of a capillary tube and the blood sample was stored in blood sample tube containing EDTA. The blood sample EDTA tubes are stored at 0-4°C for SGOT and SGPT estimation.

Histology Preparation of Liver Cystolic Microsomal Fraction:

Excised Liver of each group was bottled in 10% formalin solution. For histological studies, longitudinally cut section was taken from the left and right lobes for histological study. The samples were fixed immediately in 10% buffered formalin to prevent deformation. At least 2 slices per liver were embedded in low melting point paraffin. Specific hepatocellular lesions observed in H & E staining were recognized by light microscopy. The histological slides were coded so that the particular treatment was unfamiliar to the individual performing these studies.

Estimation of SGPT AND SGOT level in serum

Normal serum contains only small amount of GPT. Liver tissues are rich in GPT on account of these SGPT level increases markly in patients with acute hepatic disease, where liver cells are damaged. SGPT is considered a more specific index of hepatocellular damage. GPT acts by catalyzing the transfer of the amino

group from alanine to α-keto glutaric acid to form pyruvic acid and glutamic acid.

SGOT is present in abundance in cardiac muscles and also found in skeletal muscles. The SGOT level increases markedly in conditions of excessive damage to muscles especially cardiac muscles, when the GOT released from the damaged muscle escapes into the blood stream.

Statistical Calculations:

Level of significance was determined by using one way ANOVA- DMRT method of SPSS 11.0 package. The level of significance was set at $P<0.05$ and $P<0.01$.

RESULTS AND DISCUSSION

The weight variation of each group animals in one month experimental duration was observed and recorded in table 1. This study showed an abnormal increment of body weight in group C animals that were cancer control G and group A and group B animals does not have much higher increment in body weight than group C, whereas group D animals rise in body weight were normal. The survival study revealed that , a 62.5% of animals were survived in group A and group B. the group c which served as DAL control showed only 28.5% survival when compare normal mice survival as 100% after 30 days of the experiment.

Table No 1: Mean ± SD Effect of Dietary Nutrient and /or Vitamin D_3 on Body Weight at Different Stages.

Groups	**Average weight of mice at different experimental days**(in gms)				**Average Body weight** (in gms)
	Day 1	Day 9	Day 19	Day 26	
Group A	20.00	24.52	34.14	35.90	28.64 ± 2.69
Group B	20.00	25.23	36.20	37.69	29.78 ± 3.09
Group C	20.00	27.58	37.80	40.24	31.40 ± 5.846
Group D	20.00	24.10	25.25	25.50	23.71 ± 1.44

Histopathological examination in liver section from the normal group D is compared with group A, B, C which was cancer induced. The cellular architecture of hepatic lobular in Group A was normal and shows mild nuclear megali. On the contrary, Granular cytoplasm and enlarged nuclei are seen is group B. The architecture of group B is normal, and shows eosinophillic changes. Group B shows nuclear changes with no necrosis. Altered architecture can be seen in Group C, with increased nuclear size and prominent nuclei. Cytoplasm is eosinophillic and has nuclear megali in group C. Nuclear cytoplasm ratio in normal cell should be 2:1 but in cancer cell nuclear cytoplasm ratio was found to be 2:2., this above ratio might changed due to enlarged cytoplasm. From our histopathological observation in various groups, shows significant difference in cell histology of cancer induced liver and dietary nutrient rich food and /or vitamin D_3 treated cancer liver cell given table 2.

Table number 2: Effect on Dietary Nutrient and /or Vitamin D_3 on Survival Time

Groups	Survivors	Percentage Death	Day of Death	Average of survival days
Group A	5	37.5	9,11,20	22.85
Group B	5	37.5	9,14,19	23.14
Group C	2	71.43	9,11,14,20,23	19.57
Group D	7	0	Nil	30

SGOT and SGPT estimation result are elucidated in table 3 showed that the group A which are treated with dietary nutrients rich food like tomato, carrot, onion, cabbage, soy bean, and chick pea and vitamin D_3 and group B which treated only with vitamin D_3 showed decreased SGOT level and increased SGPT level than and group C (which served as cancer control). One way DMRT-ANOVA table depicts that group A

and group B which are treated with dietary nutrients rich food and / or vitamin D_3 are showed 0.05% level of significant in SGOT and 0.01% level of significant for SGPT blood samples, over group D mice blood sample (normal control) .

Table: 3: One-Way DMRT -ANOVA Table For Effect Of Dietary Nutrient Rich Foods And / Or Vitamin D_3 On Hepatic SGOT and SGPT Enzymatic Activity

Exp. Group	SGOT Average ± SD units/lit	SGPT Average ± SD units/lit
A	$295.12 \pm 33.36^{a*}$	$34.25 \pm 1.39^{a**}$
B	$271.625 \pm 9.90^{b*}$	$35.5 \pm 2.44^{b**}$
C	457.12 ± 78.26^{c}	30.42 ± 11.35^{c}
D	208.71 ± 5.018^{d}	47.428 ± 3.45^{d}
CD-0.05 CD-0.01	23.80 32.71	2.43 3.34

- Values are mean± SD of 8 animals
- Mean followed by a common superscript alphabet are not significant at 1% and 2% level by DMRT-ANOVA method.
- Statistical significance symbol: -*P<0.05 and-** P<0.01
- CD- Critical difference

Considerable changes of SGOT and SGPT were observed in group A and group B animals though they are not equal to normal control mice (group D) showed a possible preventive role of cancer in DAL bearing mice.

Our study corroborates that notion that vegetables have chemopreventive activity. Studies of our work suggest that regardless of the mechanism ,based on the results the vegetables like tomato, carrot, onion,

cabbage, soy bean and chick pea with Vitamin D_3 could be consider a potential hepatic carcinogenesis agents for mice carrying DAL, whose effect is presumably based on inhibition of growth of neoplastic cells by coordinated regulation of lifestyle surveillance, biochemical marker enzymes and morphological parameter studied herein

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