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ANTICARCINOGENIC EFFECTS OF GULGULUTHIKTHAKAM KASHAYAM AND EMILA SONCHIFOLIA EXTRACT ON TUMOUR INDUCED MICE

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ABSTRACT

The study investigated the Anticarcinogenic effect of Gulguluthikthakam Kashayam and Emilia sonchifolia extract on tumor induced mice. The study focused on plants with antineoplastic and anti-cancer activity which are not scientifically evaluated. Invitro assays were conducted in hemolysate, liver, kidney & heart and statistical analysis were done for each. A significant decrease was observed in parameters like LDH, ALP, GPT, GOT, HMG CoA Reductase, GSSG, TBARS, Total proteins, Albumin, DNA, Total lipid, Phospholipid, Cholesterol, FFA, Total hexose, Fucose, Sialic acid, WBC and Catalase, G-PX, TG, Ascorbate, Clotting Time was found to be increased in drug treated groups compared to tumor control groups. The candidate drugs show anticarcinogenic activity.

KEY WORDS: *anticarcinogenic, Gulguluthikthakam, Emilia sonchifolia, tumor induced, medicinal plants*

INTRODUCTION

Cancer is a regulatory dysfunction where the repertory of gene capabilities fails to orchestrate congruously. The life sustaining mechanism is super sophisticated and very occasionally the regulatory system fails and apoptosis is disturbed. Though several theories are available on the incidence of cancer like immunity theory, selective gene activation theory, metabolic theory, the therapeutics remain futile in later stages. Cancer cells are potentially immortal and loss contact inhibition of movement property. Alteration in surface protein enables the cancer cells to escape from the immune system and loss of cell specific adhesiveness and anchorage make them to grow in chaotic mass.

Biochemical changes constitute increased glucose consumption by cells, but O2 can't enter the membrane due to the attachment of carcinogen. In absence of O2, glucose undergoes anaerobic glycolysis and pH drops to 7- 6.5. In acidic medium, DNA losses its positive and negative radial sequences, the amino acids entering the cells, RNA changes occurs and its control mechanisms are completely lost which results in chromosomal aberration. Surface enzymes are altered. Lysosomal enzymes become toxic and leak out from the tumor mass, poison the host generating pain. New antigenic surface proteins appear which are immunologically distinct from normal cell antigens. Cancer cells secrete plasminogen activator, which dissolves intracellular matrix.

Tumor derived markers and tumor associated markers used to determine the extent of disease. They include isoenzymes, ferritins, c-reactive protein, macroglobulin. CEA determination in colonic cancer, and prostatic ACP in prostatic carcinoma. Treatment of Cancer is in stages: Curative, Palliative and Adjunctive and the common modalities are Surgery, Radiation, Chemotherapy, endocrine therapy and Immunotherapy

Many plants are used to treat cancer in ethnomedical practices in different parts of the world. About 25-30% of anti-tumor drugs are of plants origin. In Indian folklore medicine, many plants are used in the treatment of cancer which are not scientifically evaluated. New experimental studies could provide a hopeful therapy in alleviating the mystery of cancer.

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Gulguluthiktakam Kashayam is an aqueous extract of numerous medicinal plants which helps in inducing immunological surveillance. Ayurveda prescribes that continuous usage of this drug can protect the body from diseases. E. sonchifolia, herbaceous plant used in folklore against inflammation, rheumatism, tonsilitis etc. Arial parts of this plant is reported to contain flavanoids, kampferol 3-B-Dgalactose, Quercetin, ursolic acid, n-hexacosanol and triconate. Apart from its antimicrobial, antifungal and estrogenic activity., it acts as an antineoplastic agent. It helps in the induction of cell specific apoptosis and protects from DLA. Studies reveal that methanolic extract is cytotoxic towards EAC and DLA tumor lymphocytes (NHL). Flavonoids have chemo preventive role in cancer through effects of signal transduction in cell proliferation and angiogenesis.

MATERIALS AND METHODS

Experimental animals were Inbred strains of Swiss Albino male mice of 4 -5 weeks of age with body weight of 17-20g. The mice were fed stock laboratory diet and water was given water ad libitum.

The chemicals employed were of analytical reagent grade. Drug for the study: Gulguluthikthakam Kashayam' and Emilia sonchifolia extract. For in vitro and in vivo studies, DLA cell lines were used**.** Tumor cell lines were aspirated from peritoneal cavity of tumor bearing mice and washed with PBS until a white clear mass of tumor cells sediment. Cells were counted in hemocytometer. Tumor was maintained in Swiss Albino mice, of 17-20g body weight, by intra peritoneal injection of million cells in PBS (0.7ml) Palpable Ascites tumor appears within 8-12 days with a life span of 25-27 days. Swiss Albino male mice were grouped into VIII groups of 6 mice each

Group I - Normal Control-Saline
Group II -Tumor control- injected 1 x 10° cells of DLA cell line
Group III - Preventive Drug 1, -Tumor transplanted, drug I administrated after 24 hours, for 10 alternate days.
Group IV - Preventive Drug 2, -Tumor transplanted, drug 2, administrated after 24 hours, for 10 alternate days.
Group V- Curative drug 1, - Tumor transplanted, Drug 1, administered from 10^{TH} day, for 10 alternate days.
Group VI- Curative drug 2, Tumor transplanted and drug 2, administered from 10" day for 10 alternate days.
Group VII -Drug 1 control- Drug 1, alone for 10 alternate days
Group VIII- Drug 2 control -Drug 2, alone for 10 alternate days.

In vitro cytotoxicity study of the extract was studied by trypan blue exclusion method. Drug fraction that can produce 50% of the cell death in vitro was chosen as lethal dose 50 (LD50). In vivo Cytotoxicity studies, two groups of mice (6each) were selected. Group I is administered with normal saline and group II with different concentration of drug (20,40,60,80 and 100 mg) and longevity was studied.

Measurement of Tumor Development

Diameter of the mice were measured using vernier calipers and body weight of the mice on 5^{th} day, 10^{th} day,15^{th} day & 20^{th} day and average were noted.

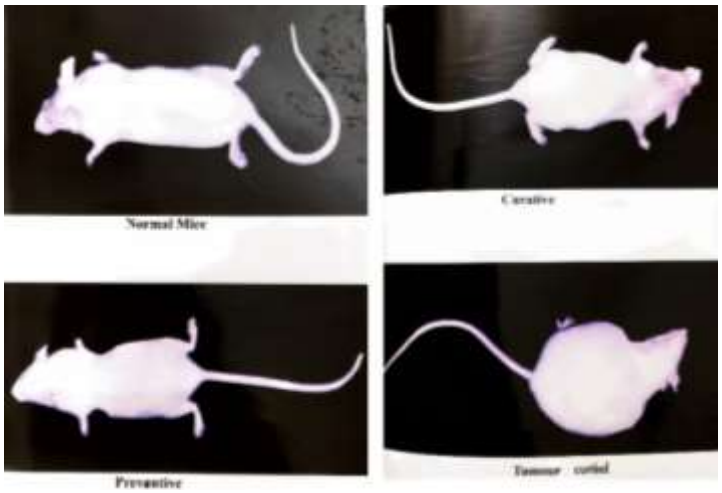

Figure 1 shows images of mice on 20^{th} day.

Collection of samples & Extraction methods

The mice were sacrificed by decapitation. Blood was collected from the jugular vein, transferred to cold containers. The RBC was washed thrice with physiological saline and made up to 6ml with distilled water. This hemolysate used for estimations

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The decapitated mice were dissected, tissues were removed, transferred to ice cold containers. A part of tissues was preserved in 10% formalin for histopathology. Extraction of tissues for different parameters were prepared using particular extraction methods.

METHODS

Alkaline phosphatase activity was estimated by the method of Kind and Amstrong, LDH by Mc Queen method, HMG COA Reductase by Rao and Ramakrishna method. Glutamate Oxaloacetate Transaminase and Glutamate Pyruvate Transaminase activity was estimated by the method of Reitman and Frankel, Catalase activity by method of Machly A et al, Glutathione peroxidase Activity by Rotruck et al, Estimation of Reduced Glutathione by Butler & Kelley. GSSG by Patterson & Lazarrow. Conjugated dienes by John et al. Ascorbate by Klin et al. DNA by Burton method. The total lipid by Phosphovanillin method, phospholipids by Silversmith and Davis. The total triglyceride by Hantzch method. Free fatty acids by the method of Falbalt et al. Estimation of total cholesterol by Zlatkis method. The method of Lowry for protein estimation. Albumin by BCG method. Fucose by Dische & Schlte. Total hexose by phenol - sulphuric acid method. Sialic acid by the method of Warren. Enumeration of WBC using Hemocytometer and Clotting time by capillary method.

STATISTICAL ANALYSIS

The data given in the tables are the mean average of the values from six rats indicated in each case as Mean +SD. Statistical significance was calculated using students t' test

Standard deviation, SD = $\sqrt{\frac{\sum(x - \bar{X})^2}{n - 1}}$

Combined standard deviation, CSD = $\sqrt{\frac{\sum(x1 - \bar{X}1)^2 + \sum(X2 - \bar{X}2)}{n1+n2}}$

't' test = $\frac{\bar{X}1 - \bar{X}2}{CSD\sqrt{\frac{1}{n1} + \frac{1}{n2}}}$

Where,
$\bar{X}1$-Mean of the first sample
$\bar{X}2$- Mean of the second sample
n1- Sample size of the first group
n2 - Sample size of the second group
A test group is compared with its control group to find out if there is any significant difference in their values between the groups.

Histopathological Results- Table 2

Normal	Tumor	Preventive	Curative
Normal tissue architecture of cells	Excessive loss of tissue architecture, necrosis, inflammatory infiltration	Normal tissue architecture, no necrotic atypia, mild inflammatory infiltrate	Few scattered inflammatory infiltrates.

Table 2 shows histopathological results of normal mice, tumor bearing mice, preventive group and curative group mice.

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RESULTS (t value and p value)

1.ALP concentration (values are expressed in mg/dl or 100mg and are mean of 6 mice + SD)

GROUP	DRUG	HEMOLYSATE t Value/ p Value	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	43.6713/<0.001	39,916|<0,001	69.6133/<0.001	408.1941/<0,001
Normal control x Drug control	GUL	16.0822/<0.001	131.5884/<0.001	I13.1376/<0.001	9.531/<0.001
Tumor control x Preventive	GUL	25.2051/<0.001	19,4889/<0.001	43.8538/<0.001	81.99/<0.001
Tumor control x Curative	GUL	23.634S/<0.001	9.4889/<0.001	34.7565/<0.001	96.56/<0.001
Normal control x Drug control	ES	000/NS	I8.57/<0,001	0.665/<0.I	1.$301/<0.1
Tumor control x Preventive	ES	29.1989/<0.001	14.8545/<0.1	I8.2535/<0.001	352.3178/<0.001
Tumor control x Curative	ES	29.8116/<0.001	13.2576/<0.001	13.7033/<0.001	72.3312/<0.001

GUL -GULGULUTHOKTHAKAM KASHAYAM, ES -EMILIA SONCHIFOLIA

2.Activity of ALT (values are expressed in IU/L and are mean of 6 mice + SD)

GROUP	DRUG	HEMOLYSATE t Value/ p Value	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	4.2212/0.001	3.9497/<0.002	4.0684<0.002	0.04716/0.1
Normal control x Drug control	GUL	170,808/0.001	25.094/<0,001	25.094/<0,001	25.094/<0,001
Tumor control x Preventive	GUL	25.094/<0,001	36.0873/<0,001	65.7972/<0.001	63.4788/<0,001
Tumor control x Curative	GUL	2.9776/<0.001	5.8348/<0,001	27.6382/0.001	23.8183/<0.001
Normal control x Drug control	ES	28.867/<0.001	68.396/<0.001	93.6349/<0.001	61.2943/<0.001
Tumor control x Preventive	ES	28.8674/<0.001	12.4202/<0,001	79.7031/<0.001	36.0723/<0.001
Tumor control x Curative	ES	31.1967/<0.001	21.5143/<0.001	42.3802/<0.001	31.5069/<0.001

3.Activity of AST (values are expressed in IU/L and are mean of 6 mice + SD)

GROUP	DRUG	HEMOLYSATE t Value/ p Value	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	361.145/<0.001	159.65/<0.001	65.8784/<0.001	36.33/<0.001
Normal control x Drug control	GUL	180.36/<0.001	5.11/<0.001	15.923/<0.001	0 /NS
Tumor control x Preventive	GUL	26.713/<0.001	17.31/<0.001	35.67/<0.001	17.31/<0.001
Tumor control x Curative	GUL	15.801/<0.001	11.57/<0.001	35.69/<0.001	4.22/<0.002
Normal control x Drug control	ES	70.189/<0.001	16.187/<001	1.3936/NS	26.5148/<0.001
Tumor control x Preventive	ES	96.8397/<0.001	14.135/<,001	16.0664/<,001	9.9853/<0.003
Tumor control x Curative	ES	35.1972/<0.001	17.187/<001	4.897/<0.001	5.4660/<001

4.Ascorbate concentration (expressed in mg/dl or 100mg and are mean of 6 mice + SD)

GROUP	DRUG	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	19.54/<0.001	5.1245/<0.002	36.0450/<0.001
Normal control x Drug control	GUL	4.1374/<0.01	1.3843/NS	9.1314/<0.001
Tumor control x Preventive	GUL	5.912/<0,001	5.1952/<0.002	26.3741/<0.001
Tumor control x Curative	GUL	0.7723/NS	0.1864/NS	21.18/<0,001
Normal control x Drug control	ES	12.61/<0.001	3.4705/<0.05	7.0364/<0.001
Tumor control x Preventive	ES	9.361/<0.001	2.23/<0.05	8.3137/<0.001
Tumor control x Curative	ES	5.2708/NS	0/NS	13.5197/<0.001

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5.Activity of GPX (Values expressed in IU/g or L and are mean of 6 mice + SD)

GROUP	DRUG	HEMOLYSATE t Value/ p Value	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal control x Tumor control	GUL	22.5602/<0.001	16.0940<0,001	18.0552/<0.001	16.6601<0.001
Normal control x Drug control	GUL	1.5827/NS	0.1664/NS	0.8851/NS	1.0745/NS
Tumor control x Preventive	GUL	16.5587/<0.001	15.1497/<0.001	12.9819/<0.001	45.1045/<0.001
Tumor control x Curative	GUL	12.0174/<0.001	15.8529/<0.001	12.2575/<0.001	25.0818/<0.001
Normal control x Drug control	ES	2.0423/NS	1.2436/NS	1.3607/NS	1.4425/NS
Tumor control x Preventive	ES	13.2875/<0.001	87.1645/<0.001	11.3914/<0.001	67.6276/<0.001
Tumor control x Curative	ES	16.4304/<0.001	27.1265/<0.001	14.2389/<0.001	61.6408/<0,001

6.Activity of GSH (Values expressed in IU/g or L and are mean of 6 mice + SD)

GROUP	DRUG	HEMOLYSATE t Value/ p Value	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	38.1943/<0.001	16.9724/<0.001	53.964/<0.001	58,0220/<0.001
Normal control x Drug control	GUL	5.2925/<0.001	4.0063/<0.01	6.0154/<0.001	6.6943/<0.001
Tumor control x Preventive	GUL	25.0052/<0.001	10.0711/<0.001	12.7392/<0.001	21.1435/<0.001
Tumor control x Curative	GUL	10.0025/<0.001	3.618/<0.01	5.1989/<0.001	8.002/<0.001
Normal control x Drug control	ES	1.8636/<0.05	2.3056/<0.05	6.3144/<0.001	9.8044/<0.001
Tumor control x Preventive	ES	20.001/<0.001	0.7072/NS	17.333/<0.001	25.4652/<0.001
Tumor control x Curative	ES	3.78 16/<0.01	0.7072/NS	3.9285/<0.01	15,4992/<0.001

7.Activity of GSSG (Values expressed in IU/g or L and are mean of 6 mice + SD)

GROUP	DRUG	HEMOLYSATE t Value/ p Value	LIVER t Value/p Value	KIDNEY t Value/p Value	HEART t Value/p Value
Normal x Tumor control	GUL	30.9732/<0,001	35.0848/<0.001	22.9419/<0,001	26.078/<0.001
Normal control x Drug control	GUL	0.2619/<0.05	2.5037/NS	0.4833/NS	0.3276/NS
Tumor control x Preventive	GUL	10.8256/<0.001	9.3186/<0.001	9.2031/<0.001	6.0021/<0.001
Tumor control x Curative	GUL	9.0542/<0.001	9.3186/<0.001	5.3741/<0.02	3.009/<0.001
Normal control x Drug control	ES	0/NS	0.6325/NS	1.2051/NS	0/NS
Tumor control x Preventive	ES	18.6756/<0.001	26.1061/<0.001	12.9412/<0.001	10.0049/<0.001
Tumor control x Curative	ES	21.6882/<0,001	20.1717/<0.001	4.4851/<0.001	3.9498/<0.01

8.Activity of CATALASE (Values expressed in IU/g or L and are mean of 6 mice + SD)

GROUP	DRUG	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	6.18/<0.001	16.0124/<0.001	12.4285/<0.001
Normal control x Drug control	GUL	2.1228/<0.05	0.0999/NS	2.8759/<0.02
Tumor control x Preventive	GUL	2.0580/<0.05	13.4900/<0.001	15.4612/<0.001
Tumor control x Curative	GUL	1.2501/0.10	1.2250/NS	2.9250/<0.02
Normal control x Drug control	ES	1.7717<0,10	1.9513/<0.10	1.5035/<0.10
Tumor control x Preventive	ES	5.5281/<0,001	3.73 15/<0.01	5.5281/<0,001
Tumor control x Curative	ES	2.5111/<0.02	5.5281/<0,001	9.2982/<0.001

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9 CONJUGATED DIENES (Values expressed in IU/g or L and are mean of 6 mice + SD)

GROUP	DRUG	HEMOLYSATE t Value/ p Value	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	8.000/<0.001	9.4149/<0.001	10.2329/<0.001	19.9718/<0.001
Normal control x Drug control	GUL	0.0002/NS	0.9905/NS	1.6344/NS	0.6853/NS
Tumor control x Preventive	GUL	10.9587<0.001	13.8698/<0.001	5.0306/<0.001	2.3808/<0.05
Tumor control x Curative	GUL	10.8745/<0.001	3.4641/<0.01	7.2375/<0.001	0.7325/NS
Normal control x Drug control	ES	1.0204/NS	1.1126/NS	1.1538/NS	1.2014/NS
Tumor control x Preventive	ES	2.3760/<0.05	23.292/<0.001	36.6815/<0.001	8.7403/<0.001
Tumor control x Curative	ES	8.63 15/<0.001	ll.6353/<0,001	47.1415/<0.001	8.6730/<0,001

10. FUCOSE (Values expressed in IU/g or L and are mean of 6 mice + SD)

GROUP	DRUG	HEART t Value/p Value	LIVER t Value/p Value	KIDNEY t Value/p Value
Normal x Tumor control	GUL	24.4975/<0.001	21.2172/<0.001	43.8602/<0.001
Normal control x Drug control	GUL	1.4145/NS	0.6332/NS	0.6342/NS
Tumor control x Preventive	GUL	26.0067/<0.001	8.1027/<0.001	26.9223/<0.001
Tumor control x Curative	GUL	10.5864/<0.001	10.6085/<0.001	18.4206/<0.001
Normal control x Drug control	ES	3.5373/NS	2.0728/NS	1.4147/NS
Tumor control x Preventive	ES	10.0015/<0.001	13.8714/<0.001	23.4093/<0.001
Tumor control x Curative	ES	8.0023/<0.001	12.0061/<0.001	22.6421/<0.001

DISCUSSION

Anticarcinogenic effect of Gulgututhikthakam kashayam and Emilia sonchifolia extract on hemolysate, liver, kidney & heart parameters are evaluated in this study. The histopathological results suggest an alternation in normal architecture of cells with increased number of cells with fatty infiltration. Tumor marker enzymes show significant changes due to metabolic alterations. Catalase and GPX activities were found to be increased in drug control group over tumor control. LDH, ALP, ALT, AST and HMG Co-A reductase were found to be decreased in drug control over tumor control group. The antioxidant enzyme also shows significant changes indicate peroxidative damage to the cells. Ascorbate and GSH concentration were found to be increased while GSSG concentration has found to be decreased in drug control over tumor control. TBARS & Conjugated Dienes have found to be decreased & increased respectively in drug control over tumor. This may be due to the increase of lipids and lipid peroxidation. A decrease in total protein and albumin were observed in drug control than tumor. DNA was found to be increased and ascorbate decreased in tumor over normal mice which may be due to hyperplasia, increased anabolism and decreased catabolism, which is reversed in drug control mice.

Lipid parameters like total lipid, phospholipid, cholesterol, FFA were observed to be decreased in drug control over tumor, while TG levels were observed to be decreased. The aberration in lipid metabolism may be due to tissue injury, metabolic disorders, deficiency of essential lipotropic factors etc. The elevation of FFA and reduction of TG may be due to increased activity of Lipase. Carbohydrate components of glycoproteins like, Total hexose, Fucose & Sialic acid were observed decreased in drug control.

Hematological parameters like Leucocyte count were found to be decreased in drug control over tumor. Hematological parameters, WBC decreased significantly in drug treated mice, whereas clotting time increased. Increase in WBC in tumor may be due to increased granulopoiesis associated with specific immune mechanism. But clotting time decreased in tumor may be due to thrombotic disorders, due to increased platelet count, platelet adhesiveness and increased clotting factors. There was significant increase in tumor diameter and body weight of the mice from 1 - 20 days as the tumor volume increases progressively.

CONCLUSION

Anti-carcinogenic effect of Gulguluthikthakam kashayam and Emilia sonchifolia extracts reduced significantly the risk of malignancy. Significant decrease observed in LDH, AST, ALT, ALP, HMG-CoA reductase, GSSG, TBARS, Total protein, Albumin, DNA, Total lipid, Phospholipid, Cholesterol, free fatty acid, total Hexose, Fucose and Sialic acid. But Catalase, GPX, GSH, Ascorbate, TG, were found to be increased in drug treated groups. Hematological parameters, WBC were found to be decreased significantly in drug treated mice, clotting time was found to be increased.

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