Ancient Science of Life, Vol No. XIII Nos. 1 & 2, July-October 1993, Pages 148 - 153

EFFECT OF *PANCHAKAVVYAM*, AN INDIGENOUS DRUG ON THE LIPID LEVELS AND RELATED ENZYMES IN FIBROSARCOMA – BEARING RATS

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Received: 12 August, 1992 **Accepted: 2 October,1992**

ABSTRACT: *In Yajur Vedhika, "Panchakavvyam", an indigenous preparation, is referred to have anticarcinogenic property. In firbosarcoma, Panchakavvyam has been tried, which on feeding promotes longevity of rats. The advantageous effect of the drug is substantial, so we tried to study the lipid changes in serum of both control and experimental animals. A significance increase in free cholesterol and phospholipids ($p<0.001$) ; total cholesterol and free fatty acids; a significant decrease in ($p<0.001$) ester cholesterol; and triglycerides. Among the enzymes, a significant increase in cholesterol ester hydrolase are observed. On panchakavvyam administration to the humour-bearing rats, a significant increase in ester cholesterol and triglycerides; and a gradual decrease in total cholesterol, free cholesterol, phospholipids and free fatty acids; and a significant decrease in cholesterol ester synthetase and an increase in the level of cholesterol ester.*

INTRODUCTION

The drug "Panchakavvyam" reported to be an anticarcinogenic agent in yajur Vedhika[24] has been made to reverse cachexia and to selectively deprive the tumour cells for catabolishing the nutrients, and the structural components of the host cells which are necessary for the normophysiology of tumour locus, by feeding a ketogenic regimen. If tumour bearing mice are provided with an exogenous supply of fatty acids, ketonemia is observed suggesting no impairment of the liver in its ability to synthesize ketone bodies[3,13].

The cachectic syndrome is characterized by depletion of the host muscle and adipose mass and a reduction in insulin secretion[7,21] accompanied by a lower serum glucose level and an elevation in serum unesterified fatty acid levels[1].

A low carbohydrate ketogenic diet might be expected to prevent host catabolism during cachexia and, in addition, reduces the rate of growth of tumours which depend on glucose for energy[22].

Normally, plasma lipid levels are governed by tissue metabolism and permeability of cell membranes to lipid constituents. While lipid changes with dietary fat modifications have been restricted to plasma, liver, aortic smooth muscles cells and fibroblasts in animals[6].

Thus panchakavvyam is capable of reversing the adverse effects of cachexia, which results in prolonging the survival time of the tumour-bearing rats.

Kumar et al have reported the changes observed in histopathology, serum marker enzymes, and plasma glycoproteins in fibrosarcoma and in treatment with panchakavvyam in rats[11]. This article deals with the effect of panchakavvyam on the lipid levels and their related enzymes in fibrosarcoma bearing rats of about 95 – 105 g.

Methods

Animals used : Adult, Wistar, male rats were obtained from King institute of Preventive Medicine, Madras, India. The pelleted animal feed marketed by M/s. Hindustan Lever Ltd., India was fed to the rats with water and libitum. The animals were housed in well ventilated, hygienic cages.

Tumer induction : Animals were transplanted with 20 – methyl cholanthrene induced cell line, 1 ml of 10 percent cell suspension containing 10 cells in physiological saline, using a 16 gauge sterile needle14 subcutaneously into the axilary region.

The drug preparation : Panchakavvyam was prepared by mixing cow's urine, cowdung (freshly voided), milk, curd and ghee in the proportion of 3:3:1.5:10:1.5 (V/V) under aseptic conditions and stored at 4^0C, throughout the experimental period. Five millilitre of the drug was given orally, for 40 days, from the 9^{th} day of transplantation, using a mouth tube.

Experimental set-up : The experimental rats were divided into 3 groups,

Group I : Normal rats

Group II : Animals transplanted with fibrosarcoma cell line

Group III :Animals transplanted with fibrosarcoma cell line and treated with Panchakavvyam.

After 40 days of drug administration, daily the animals were sacrified and the following parameters were estimated in serum of control and experimental animals.

Protein was estimated according to the method of Lowry et al[12] cholesterol ester synthetase[9], cholesterol ester hydrolase[10], total cholesterol and ester cholesterol[16], triglyceride[19], phospholipid[20] free fatty acid[8] were estimated.

For all the statistical evaluations, "student's T test" was used, to detect significant changes associated with various groups.

Results and Discussions

Depletion of the host muscle protein, glycogen and lipids accompanied by a lower serum glucose level and an elevation in free cholesterol, and a decrease in neutral fat have been reported[1]. Hence, the loss of major nutrients, particularly, excessive catabolism of lipids, leads to cachexia and the chronic and acute cachectic conditions finally leads to death in rats[21].

Table 1 shows the individuals lipid levels in both control and experimental animals. Individual lipids, namely total cholesterol, ester cholesterol, free cholesterol, triglycerides, phospholipids and free fatty acids are studied in control and experimental rats.

It is observed from table 1, a significant increase in free cholesterol and phospholipids ($p < 0.001$); total cholesterol ($p<0.01$) significance; and free fatty acid ($p<0.05$) in group II animals. Hyperlipemia

associated with cancer has been attributed to the mobilization of fat from the stores, both to meet the increased energy demands of the tumour bearing host and to supply unsaturated fatty acids for the maintenance of high concentration of phospholipids with in the tumour. The lipemia subsides terminally and it is found that the fat stores are empty[2] to meet increased caloric requirements of the host, during acute cancer condition.

In contrast, the table 1 shows a decrease ester cholesterol ($p < 0.001$) and triglycerides ($p < 0.05$) in Group II animals when compared with Group I, and significant increase in ester cholesterol ($p < 0.001$), triglyceride in Group III when compared with Group II.

Panchakavvyam treated Group III rats, also show a decrease in the levels of total cholesterol ($p < 0.05$), ester cholesterol ($p < 0.001$), free cholesterol ($p < 0.001$), phospholipids and free fatty acids in serum.

Table 2 shows the change in enzyme levels, namely, cholesterol ester synthetase and cholesterol ester hydrolase in serum of both control and experimental rats. In Group II animals, a significant increase in cholesterol ester synthetase ($p < 0.001$) and a decrease in cholesterol ester hydrolase ($p < 0.01$) is observed. In contrast, Panchakavvyam administered Group III animals, show decrease in cholesterol ester synthetase ($p < 0.01$) and gradual increase in Cholesterol ester hydrolase ($p < 0.005$).

In group II animals, CEH/CES ratio is markedly decreased when compared with Group I and Group III animals.

Elevation of plasma cholesterol mainly free cholesterol was observed in tumour induced rats. The high level of free cholesterol might have been due to low activity of lecithin cholesterol acyl transferase (LCAT) which is responsible for generating a major portions of cholesterol esters. The decreased ester cholesterol and increased free cholesterol levels in plasma indirectly aids the rapid multiplication of already proliferating cells[18]. In rat models, cholesterol levels are increased in cancer cells due to enhanced cholesterogenesis[17]. In the metabolic turnover via hexose monophosphate shunt pathway is also increased to provide NADPH (Reduced nicotinamideadenine dinucleotide phosphate) for cholesterol synthesis and ribolse phosphate synthesis for augmented nucleic acid synthesis.

The elevated level of free fatty acids could have contributed to the elevated triglyceride level in liver and kidney. The elevated plasma triglyceride level observed in tumour induced rats might have been partially due to lipoprotein lipase[15]. Stimulation of lipid peroxidation has been reported to influence lipid metabolism in the biological system which can cause tumours[4].

The synthesized cholesterol is rapidly esterified to ester cholesterol by cholesterol ester synthetase (CES), thus reducing the free cholesterol level which will otherwise inhibit the rate of limiting enzyme hydroxyl methyl glutaryl coenzyme A (HMG CoA) reductase in cancer cells[5]. CES is increased and CEH is decreased substantially in tumour induced experimental animals when compared with controls. The CEH/CES ratio is decreased. Elevation in plasma cholesterol is mediated through increased turnover and is influenced by relative balance between CEH/CES in the peripheral cells.

Increased activity of CES inside proliferating fibrosarcoma cells, may cause

increased cholesterogenesis and rapid multiplication of already proliferating cells. The above hypothesis is supported by similar observation of Rao et al[18] in acinar cell carcinoma of pancreas.

Panchakavvyam is comprised of chiefly lipid and protein and low carbohydrates. Since the drug contains high far, it supplies the fat required by the tumour – bearing animals thus preventing them from going in for cachectic syndrome and prolonging the life span.

Thus, large quantities of exogenous substrates are associated with an increase metabolic rate and stimulation of tumour growth. By increasing the lipid contribution to the nutritional regimen, it is possible to prevent the host weight loss while reducing tumour size[23].

Accordingly, the drug, panchakavvyam, which fulfills the specific requirement to suppress the tumour development nutritionally, can be considered to be having anticarcinogenic properties.

Acknowledgement

One of the authors **R.Parimala** is indebted to Madras University, India, for their financial assistance in the form of University Research Fellowship.

Table 1

Lipid levels in Serum of both control and experimental groups at the end of the experimental period. n = 6 animals in each group.

Particulars	Group I	Group II	Group III
Total Cholesterol	84.70 . 12.40	110.60 . 10.34[a@]	95.30 . 8.87[b*]
Free Cholesterol	38.09 . 5.28	97.62 . 11.94[a#]	71.43 . 9.45[b@]
Ester Cholesterol	46.61 . 6.34	12.68 . 2.38[a#]	26.87 . 3.19[b#]
Triglyceride	40.96 . 5.07	33.26 . 4.32[a*]	37.52 . 5.41
Phospholipid	1.25 . 0.18	3.02 . 0.29[a#]	2.29 . 0.21[b@]
Free Fatty acid	5.63 . 0.82	7.00 . 1.02[a*]	5.90 . 0.86

Values are expressed as Mean . SD in mg/dl of Serum.

Comparisons made between groups are:

a – Group I and II : b – Group II and III

Statistical significance : *$p < 0.05$; @$p < 0.01$; #$p < 0.001$.

Table 2

Cholesterol ester synthetase (CES) and cholesterol ester hydrolase (CEH) levels both in control and experimental period. n = 6 animals in each group.

Enzynes	Group I	Group II	Group III
Serum (Units/litre)	6.15 . 0.940	9.25 . 1.31[a@]	7.85 . 0.47[b*]
Serum CEH (Units/liter)	5.79 . 0.630	4.41 . 0.56[a@]	4.90 . 0.79
(CEH/CES) * 10^3	941.46 . 129.79	476.76 . 60.38[a#]	724.20 . 98.70[b@]

Values are expressed as Mean . SD

Enzyme units are expressed as nanomoles of cholesterol liberated or esterified/mg of protein/hour : all at 37^{o}C

Comparison made between groups are : a – Group I and II : b – Group II and III

Statistical significance : *$p < 0.05$; @$p < 0.01$; #$p < 0.001$.

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