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CHANGES OBSERVED IN SERUM MARKER ENZYMES AND PLASMA GLYCOPROTEINS IN FIBROSARCOMA AND IN TREATMENT WITH AN INDIGENOUS DRUG "PANCHAKAVVYAM" IN RATS

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ABSTRACT: *"Panchakavvyam", an indigenous preparation, has been referred to in ancient vedic literature of India, as having anticarcinogenic influence to the system, suffering from cancer. The levels of the marker enzymes such as lactate dehydrogenase and alkaline phospeatase in serum and glycoprotein levels in plasma are analysed. They are significantly reduced in the drug treated group, suggesting that this drug may have anticarcinogenic properties. Histopathological observations made on the tumor tissue of the drug-treated animals and the untreated ones, attribute concrete evidence to our inference, explaining more clearly, the observations made on the enzyme levels.*

INTRODUCTION

Cancer is a dreaded disease, in which a group of cells divide progressively in a malignant manner, without homeostatic control (Luis, 1978). Fibrosarcoma is a malignant, connective tissue tumour (Boyd. 1964). Due to fibrosarcoma, approximately 1600 deaths are reported in U.S. (NIH Report). In chronic stage, it leads to cachexia in rats and decreases in survival rate of the tomour bearing animals (Symington – Carter, 1978).

Not even a single agent, has been proved to be promising totally, in the treatment of any type of cancer till date with tolerable side effects (Marion D. Cridlan, 1978). The indigenous drug "Panchakavvyam", that is used as a sacred combination in some of the rituals of Hindu ceremonies in the Southern Parts of India (Yajur Vedhika).

The aim of this paper is to study the advantageous effects of the drug, "Panchakavvyam", rendered to the system suffering from fibrosarcoma in fighting against the malignancy. In interpreting the results of these animal experiments, one should be mindful of the fact that this drug is having anticarinogenic properties.

MATERIALS AND METHODS

Animals:

The adult, Wistar strain, male, albino rats, obtained from the King, Institute of Preventive Medicine, Madras, India, were used. The rats, weighing between 95 – 105g, initially, were housed six per cage and maintained on standard pellet diet (Hindustan Lever Ltd., India). Food and water were given *ad libitum.* No special arrangements were made for the

maintenance of temperature and light, so that the animals had a natural environment.

Tomour Induction:

Fibrosarcoma cell line, (20-methyl cholanthrene-induced) maintained in 8 – 10 weeks old, Wistar, male rats, by regular passage of 10^6 cells, subcutaneously, was used. 1.0 ml of 10 per cent cell suspension, containing the same number of cells are mentioned above in physiological saline, injected into the axillary region, used a 16 gauge, sterile needle. The transplanted tumour becomes palpable after one week. (Krishnaswamy and Purushothaman, 1980).

The drug:

The drug Panchakavvayam was prepared, by mixing five products from a cow, as described in Smirithikal Vakkiam (Yajur Vedhika). The drug was stored at 4^0C. in a sterile vessel throughout the experimental period. 5.0 ml of the drug was given orally, for forty days, from the 9^{th} day of transplantation, using a mouth tube, as a single dose. Care was taken to ingest the drug totally without any loss due to emetic reactions.

Protein was estimated according to the method of Lowry *et al* (1951). Lactate dehydrogenase was estimated by the method of King (1965). Alkaline phosphatase was estimated by the method of Moog (1946) Gamma glytamyl transpeptidase was estimated according to the method of Orlowski and Meister (1965) and modified by Rosalki and Rau (1972). Hexose was estimated by the method of Wagner (1979) and Sialic acid by the method of Warren (1959).

Experimental set up:

The rats were divided into 3 groups

Group I Control animals

Group II Animals transplanted with the Fibrosarcoma cell line i.e. suffering from the developed disease.

Group III Animals transplanted with the Fibrosarcoma cell line and which received Panchakavvyam.

After forty days of drug administration daily, the animals were sacrificed and the above-mentioned parameters were estimated.

For all the statistical evaluations, “students T test” was employed.

Histopathology

The tumour tissue pieces were removed by excision and were fixed in formal – saline. The tissues were processed, Paraffin sections obtained and stained with Haematoxylin – Eosin by the conventional methods.

Results and Discussion

The rapid increase in the turnover of malignant cells modulate the enzyme levels in blood circulation. Enzymatic changes reflect the overall changes in metabolism, that occur during malignancy (Greengard, *et. al.,* 1982). The presence of cancer may induce the release of enzymes from the surrounding normal tissue (Tietz. 1980).

Table 1 shows the levels of the enzymes such as lactate dehydrogenase, alkaline phosphatase and Gamma-glutamyl transpeptidase in serum.

When the level of the enzyme lactate dehydrogenase in control animals (Group I) are compared with the tumour bearing animals (Group I) the enzyme is significantly increased (P<0.001) in the tumour bearing group (Group II). When the tumour bearing rats (Group II), are compared the drug administered group (Group III) there is a significant decrease (p<0.01) in the level of the enzyme in the drug treated group (Group III).

Lactate dehydrogenase has been recognized as a potential tumour marker (Hill, 1957). Lactate dehydrogenase has been found to correlate well tumour mass and prognosis, in a variety of solid tumours including sarcoma (Tafafumi and Toshio Kitagawa, 1985). It has been reported that the change in lactate dehydrogenase level is more sensitive to the effect of the treatment (Ridgway, *et al* 1981).

TABLE 1

Serum Lactate dehydrogenase, alkaline phosphatase and Gamma – Glutamyl transpeptidase levels both in control and experimental groups at the end of the experimental period. Values are expressed (Mean ± S.D). The activity of the enzymes is expressed as mc. Moles of the product liberating | mg of protein | hour.
(n = 6 animals in each group)

Enzymes	Group I	Group II	Group III
Lactate dehydrogenase	890.4 ± 75	1500 *** ± 110	1250 *** ± 95
Alkaline Phosphatase	180.1 ± 18.1	250.5*** ± 21.9	214.11* ± 18.34
Gamma – glutamyl transpeptidase	1.71 ± 0.18	2.02 ± 0.23	1.99 $^{N.S}$ ± 0.22

For statistical evaluation of significant variations, Group I is compared with Group II and Group III is compared with Group III. Statistical significant alterations are expressed as

* p<0.05 **p<0.01 ***p<0.001 N.S. Not Significant

In cachexia glucose is catabolised mainly via Embden Meyerhoff pathway, increasing the turnover of blood lactate, leading to lactic acidosis (Holroyde and Reichard, 1981). It may be due to the fact that the augmented activity of the enzyme in serum as in the untreated animals (Group II) is observed. When the drug which is rich in fat is added, it may slowly reverse the adverse effects of cachexia by supplementing its fat in the place of glucose, thus reducing the level of the enzyme as in the drug treated (Group III) animals (Tisdal, *et al* 1987).

From the Table. I, the levels of the enzyme alkaline phosphatase is inferred. Alkaline phosphatase is a membrane bound enzyme. If there is rupture of membrane, then it will result in the leakage of the enzyme into the circulation, leading to an increase of that enzyme (Hilf, *et al.,* 1982). Similar picture is posed i.e the level of alkaline phosphatase is increased in the tumour significant

decrease ($p<0.05$) in the levels of the enzyme in the drug treated group (Group III) may be interpreted as follows. Some repair mechanism might have been slowly triggered in the system, posing a check in the rate of the cell destruction, and healing the ulceration.

Table 2 shows the levels of glycol-proteins such as hexose, hexosamine and sialic acid in plasma. Plasma glycoproteins are the potential tumour markers (Dacremount, 1972). When the cells undergo neoplastic transformations, it not only disrupts its cell wall, but also the nearby normal cells, which in turn show a clear view in the nature and management of the disease (Kapeller, 1976).

TABLE 2

Plasma Glycoprotein levels both in control and experimental groups at the end of the experimental period. Values are expressed as Mean ± S.D in mc.g | dl of plasma.
(n = 6 animals in each group)

Glycoproteins	Group I	Group II	Group III
Hexose	191.33 ± 15.04	281.16 *** ± 20.76	250.81* ± 19.5
Hexosamine	126.6 ± 15.95	195.25 *** ± 14.36	174.81* ± 11.67
Sialic acid	110.1 ± 11.4	210.4*** ± 15.9	180.9** ± 13.3

For statistical evaluation of significant variations, Group I is compared with Group II and Group II is compared with Group III. Statistical alterations are expressed as

* $p<0.05$ ** $p<0.01$ *** $p<0.001$

When the untreated group (Group II) is compared with the control ones (Group I), there is significant increase ($p<0.001$) in the level of the glycoproteins in the untreated group (Group II). When the drug treated ones (Group III) are compared with the untreated ones (Group II), there is a significant decrease ($p<0.05$) in the level of the glycoproteins in the drug treated group (Group III). Sialic acid is much significantly reduced ($p<0.001$) in the drug treated group (Group III).

The levels of the glycoproteins correlate well correspondingly with the tumour burden (Silver *et al.*, 1979). From the table it leads us to hypothesise that the drug brings back normophysiology of the affected cells from their altered pathophysiology.

Histopathology

Fig. A shows the histopathological changes in the tumour tissue of the untreated group (Group II). It has spindle shaped cells with hypochromatic nucleus, amphophilic cytoplasm with increased mitotic activity.

Fig. B and C show the different regions of the drug administered tumour tissue (Group III). The picture shows marked regenerative changes in the drug treated ones (Group III) when compared with the untreated ones (Group II).

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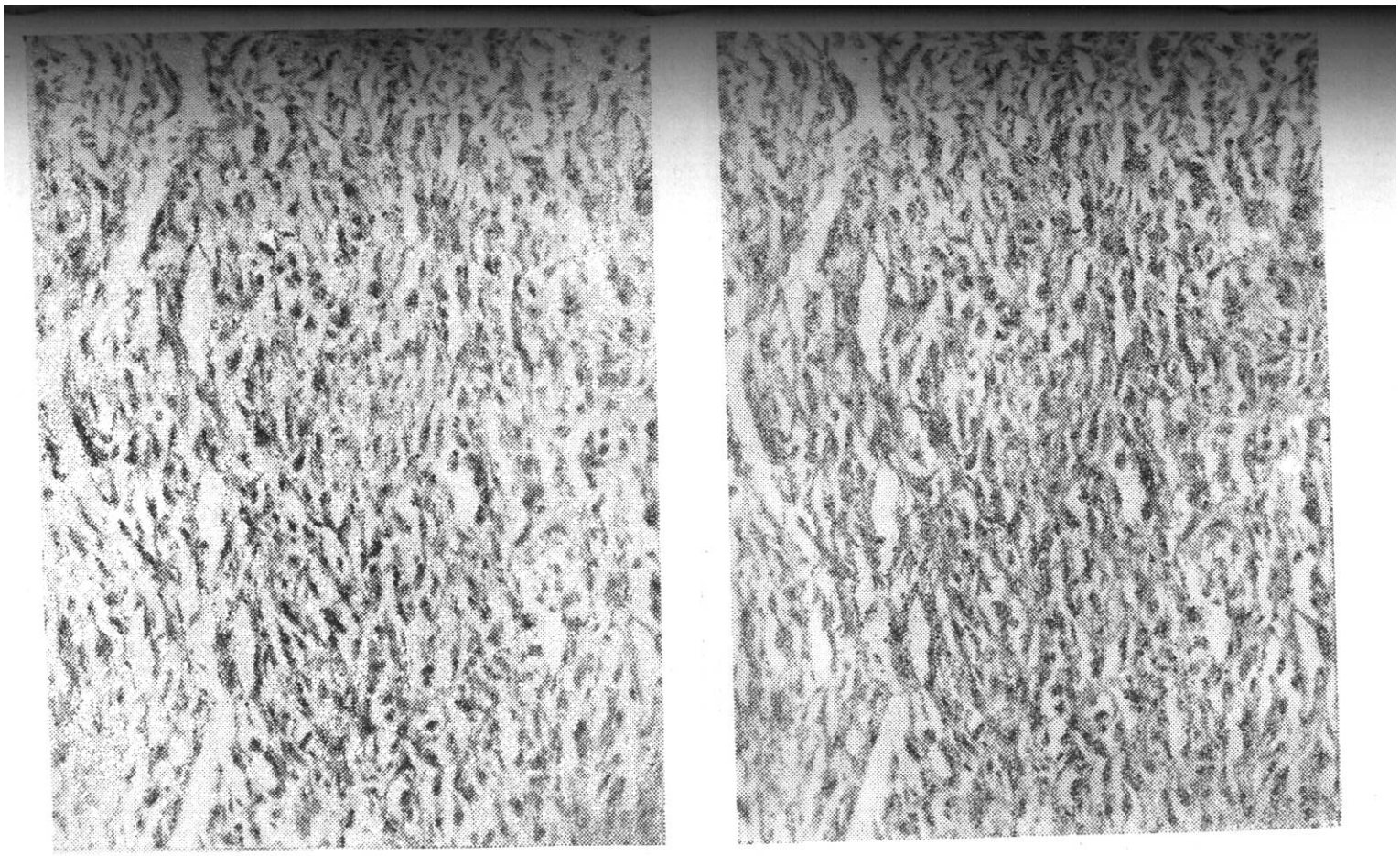
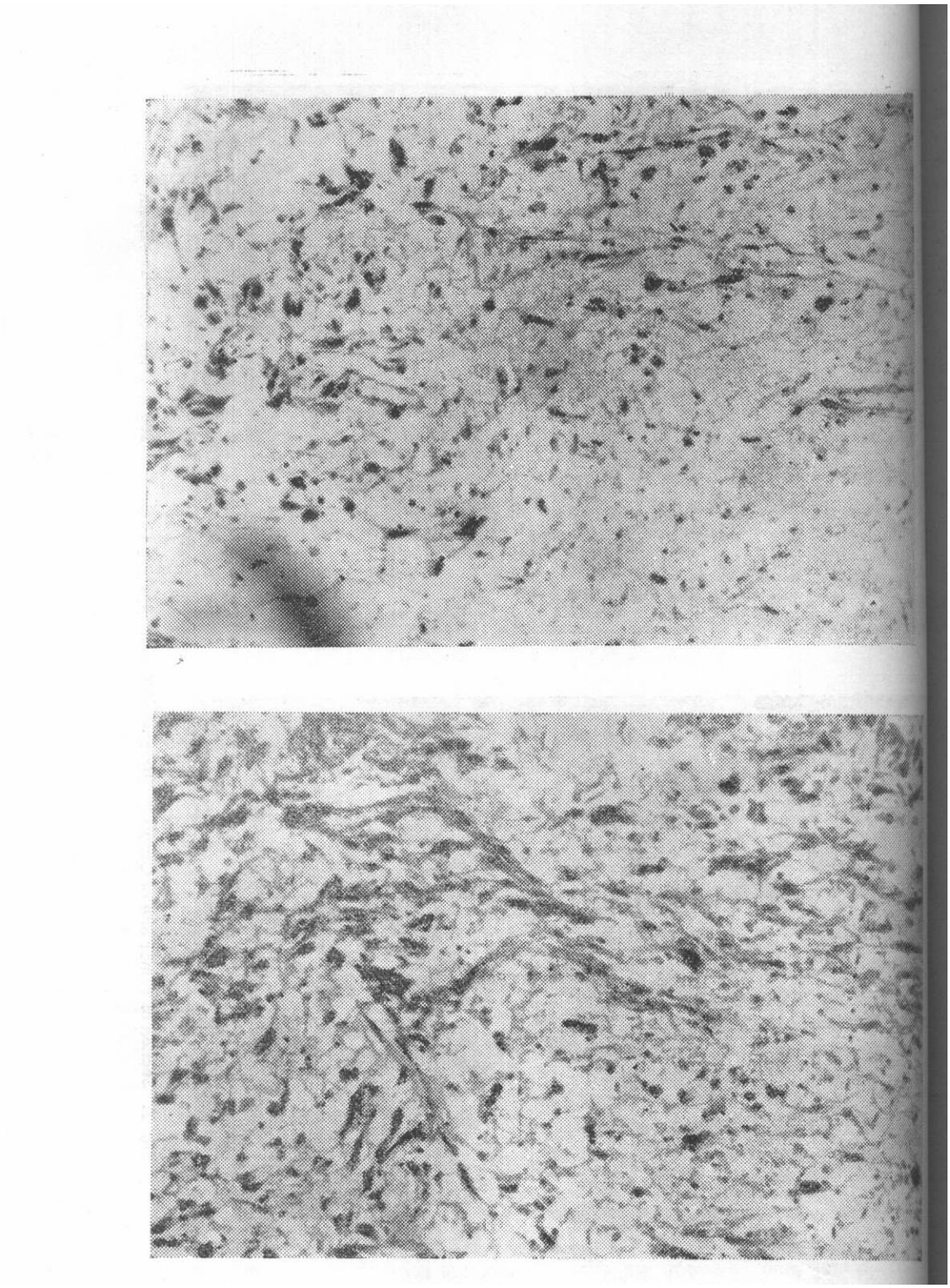


Fig. A Section of Tumor Site of Group II (Untreated Tumor Bearing Group) 200 × Haenotoxylin—Eosin



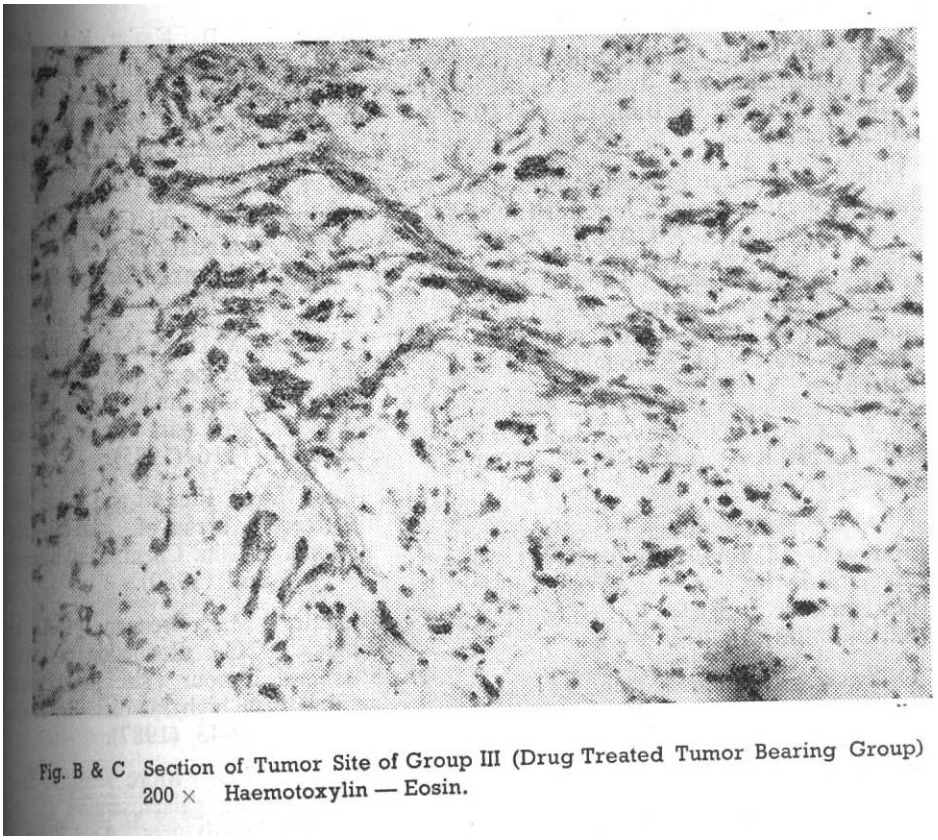


Fig. B & C Section of Tumor Site of Group III (Drug Treated Tumor Bearing Group) 200 × Haemotoxylin — Eosin.

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