Biochemical Effects of Plumbagin On Fibrosarcoma Induced Rats

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Abstract

Natural products from plants are used for treating a number of diseases. Many of the pharmacological principles of the currently used anticancer agents have been initially isolated from plants. Plumbagin, a napthaquinone derivative from Plumbago zeylanica and has been claimed to possess antitumor effect. The tumor weight was found to be reduced in methylcholanthrene induced fibrosarcoma rats after plumbagin treatment. Elevated levels of proteins, lipid profile and also in the activities of pathophysiological enzymes such as gamma glutamyl trans peptidase (GGT), lactate dehydrogenase (LDH), glutamate oxaloacetate transaminase (GOT), glutamate pyruvate transaminase (GPT), in plasma, liver and kidney extracts of fibrosarcoma rats decreased significantly after Plumbagin treatment. These observations clearly suggested the antitumor potency of plumbagin in experimentally induced fibrosarcoma in rats.

Key words: Plumbagin, lipid profile, fibrosarcoma, pathophysiological enzymes

Introduction

Since antiquity human beings are suffering from variety of diseases, out of which, cancer is the second most common cause of death after the cardiovascular diseases. Human of all ages develop cancer and a variety of organs are affected. Sarcoma, are tumors made up principally of connective tissue cells, which are of mesodermal origin[1]. Fibrosarcoma is still best defined as a malignant tumor of fibroblasts that shows no other evidence of cellular differentiation and is capable of recurrence and metastasis. Electron microscopically the tumors are largely composed of elongated fibroblast like cells having irregularly outlined nuclei, infrequent nucleoli and prominent rough endoplasmic reticulum, which is often dilated and contains granular or amorphous material[2].

Plants are a suitable source for therapeutics. So far, many products have been found to be active antitumor agents both in animal and human tumor[3]. Natural products from plants are rich sources used for treating a number of diseases. Many of the pharmacological principles of the currently used anticancer agents have been initially isolated from plants. Plumbagin, a napthaquinone derivative from Plumbago zeylanica were reported to have antitumor activity on rat fibrosarcoma. A prime goal of experiments with animal models is to further understanding of diseases of man and ultimately to provide information which can serve as a basis for their prevention and treatment. Hence, this study is designed to assess various biochemical alterations in fibrosarcoma and also to understand the antitumor effects of Plumbagin.

Materials and Methods

Plumbagin was purchased from Sigma (St. Louis,MO). Stock solution of plumbagin was made in dimethyl sulphoxide (DMSO) (Sigma). All other chemicals used were of analytical grade.

Male Wistar rats weighing approximately 250g were housed in solid-bottomed polypropylene cages under strict veterinary supervision and maintained in control rooms with 12 h light/dark cycle. The animals received commercial rat diet and water *ad libitum*. This study conformed to the guiding principles of Institutional Animal Ethical Committee (IAEC), Committee for the purpose of Control and Supervision of Experiments on Animals (CPCSEA) and the Guide for the care and use of laboratory animals.

Experimental design and treatment protocol

Induction of experimental fibrosarcoma.

Fibrosarcoma was induced in rats by the method of Nagarajan and Sankaran[3]. Fibrosarcoma cell lines obtained from induced rats were transplanted under aseptic conditions. The tumor tissue was used for transplantation after the removal of necrotic tissue. A suitable bit of the tumor was minced into fine fragments suspended in physiological saline. A 10 % suspension (0.5 ml) of tumor tissue was injected into the auxiliary and inguinal regions through the usual puncture technique. The transplanted tumor takes a week to become palpable, grew upto the end of a week. The animals were broadly divided into two groups:
Group 1: Control (received saline only); Group 2: Induced experimental fibrosarcoma. Animals of group 2 were further divided into experimental fibrosarcoma at the 20^{th} day and 30^{th} day.
Group 3: Experimental fibrosarcoma at the 20^{th} day and 30^{th} day + echitamine chloride. (Plumbagin, was dissolved in 0.9% saline was injected subcutaneously (10mg/kg bodyweight). Each group comprised 6 animals. After the experimental period, the animals were sacrificed by cervical decapitation and blood was collected for the biochemical investigations. The organs were blotted and cut them into pieces before weighing. 100mg of the tissue was weighed and homogenized in 0.1 M Tris HCl buffer (pH 7.4). The tissue homogenates were centrifuged and the supernatant was taken for assays.

Biochemical assays

Protein in serum was estimated by the method of Lowry *et al*.,[4] using crystalline bovine serum albumin as the reference standard. Enzyme markers such as gamma glutamyl transferase[5], lactate dehydrogenase[6], aspartate amino transferase[7], alanine amino transferase[7], cholesterol[8], triglycerides[8] and phospholipids[9] were determined in serum, liver and kidney tissues.

Statistical methods

All values used in analysis represented as mean ± SE of 6 rats.

Results and Discussion

The level of total protein in serum, liver and kidney of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with Plumbagin, is shown in Table 1. Increased protein content in serum and tissues of fibrosarcoma rat in progressing order were noticed. In tumor bearing animals there is an increase of protein content in liver[10]. In our studies the increased protein content is found to be decreased in serum, liver and kidney during the 20 days treatment of plumbagin. Though the treatment of plumbagin, is not able to correct the protein content to normal, it can be appreciated that the drug therapy could able to check the elevation of the protein content and also reduces to certain extent.

Table 1. Total protein content in serum (g/dl), liver and kidney (mg/g of wet tissue) of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with Plumbagin (EC). The values are expessed as mean ± SD (N = 6).

	Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with EC	
		20^{th} day	30^{th} day	20^{th} day	30^{th} day
Serum	7.01 ± 0.24	8.01 ± 0.21	9.21 ± 0.32	7.52 ± 0.18	8.21 ± 0.16
Liver	194.7 ± 10.2	204 ± 9.4	225 ± 8.7	199 ± 7.9	202 ± 7.6
Kidney	173 ± 11.1	178.2 ± 8.3	198.1 ± 7.5	176.2 ± 9.1	179 ± 8.2

The activity of gamma glutamyl transpeptidase in serum, live and kidney of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with plumbagin, is shown in Table 2. There is progressive increase in the activity of gamma glutamyl transpeptidase in serum (54 %), liver (51 %) and kidney (46 %) after 30 days in fibrosarcoma induced rats[11]. The gamma glutamyl transpeptidase is reduced to near normal in sarcoma treated with plumbagin. This may be due to tumor regression.

Table 2. Activity of Gamma GT in serum (IU/L), liver and kidney (units/ mg of protein) of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with EC. The values are expessed as mean ± SD (N = 6).

	Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with EC	
		20^{th} day	30^{th} day	20^{th} day	30^{th} day
Serum	9.71 ± 0.71	10.32 ± 0.62	12.52 ± 0.75	9.43 ± 0.63	10.29 ± 0.63
Liver	0.36 ± 0.01	0.40 ± 0.02	0.55 ± 0.03	0.38 ± 0.02	0.42 ± 0.016
Kidney	3.52 ± 0.3	4.17 ± 0.20	5.32 ± 0.23	3.90 ± 0.21	4.20 ± 0.21

Table 3 depicts activity of lactate dehydrogenase (LDH) in serum, liver and kidney of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with . Plumbagin,. A consistent rise in LDH activity has been observed in serum, liver and kidney of fibrosarcoma induced rats at the 30^{th} day[12]. This might be due to an increasing predominance of anaerobic glycolysis in fibrosarcoma rats at a faster rate. So that LDH would be expected to reflect tumor activity. The elevation of LDH is due to the over production of the enzyme by the tumor, tumor blockade of the duct system through which enzymes passes, changes in the permeability of cell allowing leakage of soluble enzyme into circulation, status of kidney functions where homeostasis mechanisms involve urinary excretion of the enzyme and possible presence of abnormal concentrations of endogenous and exogenous imhibitors or activators. The therapeutic effect of plumbagin, is well proved in the present investigations where it effectively brought back the serum, liver and kidney LDH levels to near normal levels. This may be due to reduced aerobic glycolysis, when the tumor gets regressed[13].

Table 3. Activity of lactate dehydrogenase in serum (IU/ L), liver and kidney (μ moles of pyruvate formed / mg of protein / minute) of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with EC. The values are expessed as mean ± SD (N = 6).

	Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with EC	
		20^{th} day	30^{th} day	20^{th} day	30^{th} day
Serum	311 22.4	371.2 ± 20.45	439 ± 19.6	335 ± 21.6	365 ± 18.7
Liver	1270 ± 31	1511 ± 42	1667 ± 25	1495 ± 37	1445 ± 47
Kidney	1601 ± 58	1643 ± 46	1820 ± 50	1637 ± 47	1698 ± 61

Table 4. Activities of aspartate amino transferase (GOT) and alanine amino transferase (GPT) in plasma , liver and kidney of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with . Plumbagin,. The activity of both transaminases was studied in serum of nomal persons and of patients with a wide variety of cancer[10]. The present imvestigation indicates increased transaminases activities in serum and considerably decreased in liver of fibrosarcoma induced rats. The activity of transaminases in plasma is found to be reduced, whereas in liver, the reduced activity is increased during the 10 days treatment. However the decreasing activities in serum and the increasing activities in liver after 20 days treatment, is greater than that of 10 days treatment, which clearly shows a good controlling capacity of plumbagin, over the alterations of transaminase activities.

Table 4. Activities of aspartate amino transferase (GOT) and alanine amino transferase (GPT) in plasma (IU/L), liver and kidney (n moles of pyruvate liberated / hr /mg protein) of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with EC. The values are expessed as mean ± SD (N = 6).

Enzymes		Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with EC	
			20^{th} day	30^{th} day	20^{th} day	30^{th} day
GOT	Plasma	14.8 ± 1.0	15.5 ± 2.0	17.1 ± 2.0	15.4 ± 1.1	16.8 ± 1.0
	Liver	611 ± 42	590 ± 15	572 ± 22	592 ± 15	582 ± 2.0
GPT	Plasma	10.9 ± 0.6	13.9 ± 1.3	17.8 ± 2.3	13.7 ± 1.1	16.7 ± 1.4
	Liver	958 ± 30	937 ± 26	901 ± 25	938 ± 28	915 ± 22

The lipid profiles such as cholesterol, triglycerides and phospholipids are estimated in serum, liver and kidney of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with Plumbagin, is given in Tables 5,6 and 7. Raja[13] noticed low cholesterol level in cancer patients. It has been suggested that low concentration of total cholesterol associated with increased cancer risk indirectly by virtue of their association with low concentration of carotene or retinol. Cholesterol level is reduced in serum, liver and kidney, while the concentration of triglycerides and phospholipids are increased noticeably in the present investigations.

Table 5. Cholesterol content in serum (mg/dl), liver and kidney (mg/g of wet tissue) of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with EC. The values are expessed as mean ± SD (N = 6).

	Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with EC	
		20^{th} day	30^{th} day	20^{th} day	30^{th} day
Serum	82.5 ± 2.5	93.5 ± 1.5	108.4 ± 5.5	77.2 ± 2.9	82.5 ± 4.7
Liver	6.12 ± 0.5	5.72 ± 0.2	5.0 ± 0.1	5.8 ± 0.1	5.7 ± 0.17
Kidney	7.30 ± 0.3	6.70 ± 0.1	6.01 ± 0.25	6.81 ± 0.1	6.75 ± 0.17

Table 6. Triglycerides content in plasma (mg/dl), liver and kidney (mg/g of wet tissue) of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with EC. The values are expessed as mean ± SD (N = 6).

	Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with EC	
		20^{th} day	30^{th} day	20^{th} day	30^{th} day
Plasma	65.5 ± 5.3	75.1 ± 2.1	84.5 ± 5.1	71.2 ± 1.2	74.3 ± 2.6
Liver	2.80 ± 0.75	3.90 ± 0.21	5.01 ± 0.31	3.40 ± 0.15	3.8 ± 0.2
Kidney	1.6 ± 0.18	2.1 ± 0.15	2. 7 ± 0.16	2.0 ± 0.16	2.1 ± 0.01

Table 7. Phospholipids content in plasma (mg/dl), liver and kidney (mg/g of wet tissue)of control, fibrosarcoma induced rats and fibrosarcoma induced rats treated with EC. The values are expessed as mean ± SD (N = 6).

	Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with EC	
		20^{th} day	30^{th} day	20^{th} day	30^{th} day
Plasma	104 ± 13	107 ± 12.1	129 ± 7	103 ± 11.2	118 ±5.6
Liver	17.45 ± 3.62	19.3 ± 3.1	24.5 ± 2.5	18.2 ± 2.6	20.1 ± 2.0
Kidney	14.67 ± 0.83	15.70 ± 0.74	16.3 ± 0.52	15.23 ± 0.43	15.7 ± 0.35

In the tumor-bearing animals, increased lipolytic activity was reported, associated with hyperlipidaemia[3]. The hyperlipidaemic state was found to correlate directly with tumor burden and was reversible on tumour removal or remission. significantly attenuated these alterations of lipid levels in tissues as well as in serum. The depletion of fat stores associated with tumour growth indicates high energy requirement and hence increased lipolytic activity[14]. The normalisation of lipid levels in tissues and serum upon plumbagin treatment may be due to enhanced lipogenesis or decreased lipolysis or both. In fact, earlier studies have indicated the lipogenic properties of plumbagin[15].

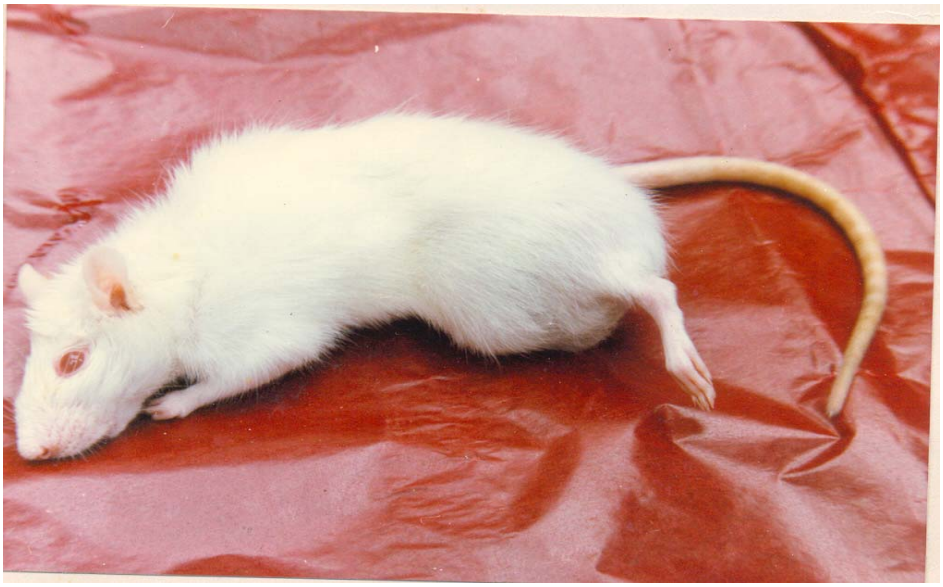
Triglycerides pool was increased by 50 % in tumor bearing rat compared to controls[3]. These observations are in accordance with our observations on the levels of cholesterol, triglycerides and phospholipids in fibrosarcoma induced and drug treated rats. The accumulation of triglycerides in fibrosarcoma condition suggests that there should be some diversion of fatty acids from the oxidative sequences to the esterfication pathway. From the results, it may be concluded that the drug effectively alter the changes occurring in induced rats with respect to lipid profiles.

The biochemical effects that are reflected on the growth of fibrosarcoma are very much reduced after the treatment of plumbagin. From the above observations it is clear that the drug would influences in all possible ways to nullify the unnatural events that are happened during the growth of fibrosarcoma and have indicated the antitumor effect of plumbagin[16].

Plate 1 (a) represents the fibrosarcoma induced rat. The progressive growth of growth of tumor in plate 1(a) is reduced greatly after plumbagin, treatment in plate 1(b) which provides further evidence for the anticancer property of plumbagin.

Plate 1(a) Fibrosarcoma induced rat

Plate 1(b) Fibrosarcoma induced rat treated with plumbagin.



Conclusion

The overall observations clearly exhibited the biochemical alterations during the growth of fibrosarcoma and the powerful regressive potency of plumbagin.

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