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ANTI OXIDANT PROPERTY OF PLUMBAGIN ON FIBROSARCOMA INDUCED RATS

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Abstract

Enhanced generation of reactive oxygen/ nitrogen species (ROS/RNS) and the ensuing phenomenon in the form of oxidative stress have been implicated in the etiology of cancer. Anti oxidants due to their ability to neutralize the toxic free radicals or damage induced by them have potential applications in the prevention and/or therapy of human ailments. Plumbagin, a napthaquinone derivative from *Plumbago zeylanica* and has been claimed to possess antitumor effect. Elevated levels of lipid peroxides and decreased activities of antioxidant enzymes such as catalase, superoxide dismutase and Glutathione peroxidase were assayed in plasma, liver and kidney of fibrosarcoma induced rats, and the levels significantly reverted back to normal, after treatment with plumbagin. These observations clearly suggested the antitumor potency of plumbagin in experimentally induced fibrosarcoma in rats.

Keywords: Antioxidant enzymes, Fibrosarcoma, Lipid peroxidation

Introduction

Fibrosarcoma is a malignant neoplasm (cancer) of mesenchymal cell origin in which histologically the predominant cells are fibroblasts that divide excessively without cellular control; they can invade local tissues and travel to distant body sites. Fibrosarcoma is part of a larger collection of cancers known as sarcomas. Sarcomas are spindle cell malignancies of mesenchymal cell origin and are named and classified after the predominant cell line that is present. For example, in bone (osteosarcoma), cartilage (chondrosarcoma), smooth muscle (leiomyosarcoma), and skeletal muscle (rhabdomyosarcoma). Though all are sarcomas, specific diseases vary considerably in presentation, treatment, and prognosis. At the same time, there are characteristics shared by many sarcomas. Since, all are connective tissue in origin, they form solid tumors, as opposed to a disease such as leukemia in which abnormal cells are circulating in the blood stream, or certain gastrointestinal tract cancers that may develop within the wall of a hollow organ[1].

An ever increasing number of pharmacological effects have become known through the discovery of new herbal drugs with variations in chemical structure and related derivatives. Most of the herbal drugs are a mixture of a number of plant ingredients. So far, many products have been found to be active antitumor agents both in animal and human tumor[2].

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 the antioxidant status of 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 confirmed 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 Jayamathi *et al*[3]. A suitable bit of the tumor was

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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 up to 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 20th day and 30th day.

Group 3: Experimental fibrosarcoma at the 20th day and 30th day + plumbagin (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

Lipid peroxide[4], anti oxidant enzymes such as catalase[5], superoxide dismutase[6] glutathione peroxidase[7] were assayed in serum, liver and kidney tissues. Protein in serum was estimated by the method of Lowry *et al*.[8], using crystalline bovine serum albumin as the reference standard.

Statistical methods

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

Results and Discussion

The rate of formation of malondialdehyde (MDA), the end product of enhanced peroxidation of arachidonic acid (lipid peroxidation) and the levels were increased in fibrosarcoma bearing rats when it is compared with respective controls. The increase in malondialdehyde levels might be associated with the higher production of ROS. It is well known that lipid oxidation and ROS are implicated in carcinogenesis and might be etiologically involved in the promotional phase[9]. In plumagin treated animals, the corrected level of these parameters were observed likely to near normal values showed significant protective effect (Table-1).

Table 1. Levels of lipid peroxide in serum, liver and kidney of normal, fibrosarcoma induced rats and fibrosarcoma induced rats treated with plumagin

		Control	Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with plumagin	
			20th day	30th day	20th day	30th day
Lipid peroxide	Serum	2.32 ± 0.32	2.43 ± 0.36	2. 52 ± 0.4	2.40 ± 0.28	2.48 ± 0.28
	Liver	138.2 ± 11.7	159 ± 10.5	198 ± 9.5	152 ± 8.32	179.5 ± 8.17
	Kidney	75 ± 4.6	90.6 ± 3.5	115.2 ± 4.0	85.2 ±2.8	94.7 ± 3.2

Lipid peroxide in serum is expressed as n mol of malonyldialdehyde /ml; in liver and kidney as n moles of malonyldialdehyde / 100 mg protein .The values are expressed as mean ± SD (N = 6).

Table 2. Activity of catalase, Super oxide dismutase, Glutathione peroxidase (GPx), and in liver and kidney of normal, fibrosarcoma induced rats and fibrosarcoma induced rats treated with plumagin

			Fibrosarcoma induced rats		Fibrosarcoma induced rats treated with plumagin	
		Control	20th day	30th day	20th day	30th day
	Liver	67.5 ± 5.1	58.6 ± 4.8	55.5 ± 3.2	59.5 ± 5	60.5 ± 3.2
Catalase	Kidney	7.01 ± 0.62	5.25 ± 0.12	3.6 ± 0.2	5.65 ±0.3	5.8 ± 0.5
Super oxide	Liver	6.42 ± 0.7	4.95 ± 0.5	3.5 ± 0.6	5.15 ± 0.4	5.34 ± 0.5
dismutase	Kidney	3.18 ± 0.2	2.4 ± 0.1	1.75 ± 0.15	2.48 ±0.3	2.59 ± 0.1
	Liver	86.2 ± 5.4	74.2 ± 2.8	61.3 ± 2.5	76.5 ± 3.0	72.1 ± 2. 0
GPx	Kidney	43.3 ± 4.7	34.3 ± 3.0	28.7 ± 2.7	36.1 ± 2.0	36.5 ± 2.1

Catalase (CAT), Super oxide dismutase (SOD) and in liver and kidney as n moles of malonyldialdehyde / 100 mg protein. One unit of SOD activity is the amount of protein required to give 50% inhibition of adrenaline autooxidation. CAT activity is expressed as n mol of hydrogen peroxide decomposed / min / mg protein. GPx as n moles of glutathione oxidized/ min/mg protein of normal, fibrosarcoma induced rats and fibrosarcoma induced rats treated with plumagin. The values are expressed as mean ± SD (N = 6).

Antioxidants are substances that prevent damage to cells caused by free radicals. Free radicals are molecules that have lost an electron, thus are unstable. These free radicals basically steal electrons from other molecules in effort to heal themselves, ultimately creating new free radicals in the process. By stealing electrons, it can cause damage to DNA, leading to the possible development of cancer[10]. The antioxidant

mechanisms are the evolutionary designs that avidly react and annihilate ROS before they inflict oxidative damage to tissues and cells ROS can cause DNA and protein damage, initiate lipid peroxidation, oxidize α1-antitrypsin and stimulate the release of proinflammatory cytokines[11].

Enzymatic antioxidants such as catalase, SOD and GPx in serum liver and kidney were decreased significantly in fibrosarcoma induced rats whereas plumagin treated groups were found to have significant protective effect as reflected by an increase in the levels of antioxidants as shown in Table 2. In plumagin treated animals, the corrected level of these parameters were observed likely to near normal values. Level of lipid peroxide was observed with concomitant decreases in the level of enzymatic antioxidants in fibrosarcoma bearing rats when compared with control animals.

The present findings demonstrated that the reduced activities of catalase, SOD and GPx, were well correlated with an increase in MDA in fibrosarcoma bearing rats with respective controls, which are the products of enhanced peroxidation of arachidonic acid[12]. These observations clearly suggested the antitumor potency of plumagin in experimentally induced fibrosarcoma in rats. Therefore, attention has been focused on studying the various plant ingredients in the drug as a whole for its antitumor effects.

Conclusion

Thus, plumagin can be accepted to pose antioxidant mechanism against excessively formed reactive species due to impaired antioxidant systems in fibrosarcoma conditions that cause membrane damage leading to deleterious effects.

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