INTRODUCTION

Cancer is a major health hazard in the world and claims over six million lives every year (Abdullaev, 2001). Cancer, a term comprising over 100 types of malignancy, is one of the major burdens of chronic disease in the world. It is very difficult to cure this disease because of its multifactorial etiology. Early in the 20th century, only cancers small and localized enough to be completely removed by surgery were curable. Later, radiation was used after surgery to control small tumor growths that were not surgically removed. Finally, chemotherapy was added to destroy small tumor growths that had spread beyond the reach of the surgeon and radiotherapist [1,2]. Plant derived substances have recently become of great interest in owing to their versatile applications. Medicinal plants are the richest bioresources of drugs of traditional systems of medicine, nutraceuticals, food supplements, folk medicines, pharmaceuticals intermediates, and chemical entities for synthetic drugs [3]. The medicinal value of plants lies in some chemical substances that produce a definite physiological action on the human body. Many of the indigenous medicinal plants are used as spices [4]. With the aim of searching novel compounds without undesirable side effects, we focused on natural medicines. Plants are reported to have a long history in the treatment of cancer [5]. The use of plants and plant-based products for cancer treatment is rapidly growing in medical practices [6].

Scientific advancements have led to the exploration of many such plants chemically for its constituents and their impact in treating several types of diseases. As little attention has been devoted to understand the functional role of ethno-medicinally important plant proteins in relation to its therapeutic use and considering the potential advantage of using plant proteins as drugs (due to nontoxic nature), a through scientific research is attempted. Proteome analysis deals with determination of protein compositions of different samples. The objective of this study is to assess the antitumor and antioxidant activity of Td in mice model.

METHODS

Collection and authentication of plant

The fresh leaves of Td (Apocynaceae) were collected from Coimbatore district, India. Taxonomic authentication was done by Dr.G.V.S. Murthy, Taxonomist, TNAG, Coimbatore, Tamil Nadu, India, and the authentication number BSI/SRC/5/23/2015/Tech/2083.

Preparation of protein extract of Td leaf (TdPf)

Protein was extracted by recrystallization of ammonium sulfate. 20% fresh leaves of Td were taken and homogenized with phosphate-buffered saline buffer pH 7.2 and were centrifuged at 5000 rpm for 10 minutes. Pellets were discarded and supernatant was saved. To the supernatant saline buffer pH 7.2 and were centrifuged for 5000 rpm for 10 minutes. Supernatant was saved and centrifuged for 10 minutes. Pellets were discarded and supernatant was saved. To the supernatant saline buffer pH 7.2 and supernatant was saved. The supernatant was dialyzed against 0.1 M phosphate buffer, pH 7.2 at 4°C for 10 minutes. The supernatant was discarded and the pellet was suspended with dialysis membrane for salting out. The crude extract was kept at −20°C.

Preparation of TdPf extract

The protein extract of Td animals restored the antioxidant enzymes when compared to the mice of the DLA control group.

Results:
The results showed that the protein extract of Td animals restored the antioxidant enzymes when compared to the mice of the DLA control group.

Conclusion: The findings indicate that the extract of plant protein has antitumor activity by preventing the lipid peroxidation and thereby promoting the antioxidant systems in DLA induced mice. And hence, it is evident that these extract could be a natural anticancer agent for the human health.

Keywords: Dalton’s lymphoma ascites, Antitumor, Tabernaemontana divaricata, Lipid peroxidation antioxidant.
Antioxidant assays

The liver homogenate was used to analyze the enzymatic antioxidant activities by superoxide dismutase (SOD), catalase (CAT), glutathione (GSH) peroxidase (GPx), and nonenzymatic antioxidants such as vitamin A, vitamin E, and reduced GSH were evaluated in the liver tissue homogenate using standard kit methods.

Histopathological study

The liver tissue samples collected were fixed in 10% formalin solution. After fixation, the tissues were embedded in paraffin and sections cut at 5 µm to later be stained with hematoxylin and eosin. The sections were then examined under light microscope and photographed (Standish et al., 2006).

Statistical analysis

The results obtained were reported as mean ± standard deviation. One-way and two-way analysis of variance was performed to analyze statistically significance of the data using Agres Statistical Package.

RESULTS

Estimation of liver marker enzymes in serum samples

Graph 1a-c shows the activities of marker enzymes in control and lymphoma-bearing animals, respectively. In lymphoma-bearing animals, the activities of the aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were significantly (69.40±2.48; 82.87±4.96) increased, whereas ALP were found to be significantly (56.5±3.8; 40.02±1.28; 8.97±1.33) increased when compared to the control mice (p<0.05). All the marker enzymes were found to be significantly increased in the serum of tumor-bearing animals when compared to normal control mice. On TdPF treatment, the activities of all these enzymes were significantly brought back to near normal levels.

Estimation of lipid peroxidation

This study shows that a significant increase in lipid peroxidation (Graph 2) was observed in DLA-induced mice.

Estimation of antioxidant activity

Enzymatic antioxidant

DLA cells induced mice showed a significant (p<0.05) decreased levels of SOD (0.83±0.03), CAT (1.93±0.03), and GPx (0.99±0.01). The treatment of TdPF alone at 5.2 µg/kg body weight showed a significant increased levels of SOD (1.74±0.46), CAT (1.94±0.03), and GPx (0.99±0.01) when compared to the normal mice group and also highly significant in 60 days of treated animals (p<0.05) (Table 1).

Estimation of nonenzymic antioxidants

Table 2 exhibits the levels of nonenzymic antioxidants in the liver of control and experimental animals. From the Table 2, it is found that the level of vitamin A, vitamin E, and GSH was significantly reverted back to near normal level when treated with the plant extracts when compared to Group III treated (standard antioxidant).

Histopathological observations in the liver of TdPF-treated mice were comparable to that of controls and silymarin without any structural changes in the liver morphology.

Plate 1a-j indicates the histopathological observation of DLA-bearing mice. (a) Untreated; (b) paraffin oil 15 days observation; (c) silymarin (standard drug); (d) protein extract (TdPF); (e) protein extract + DLA; (f) paraffin oil 60 days observation; (g) silymarin 60 days observation; (h) plant extract 60 days; (i) plant extract + DLA 60 days; (j) DLA 15 days observation.

Hepatic cells of DLA-induced mice showed large confluent areas of hepatocellular necrosis with peripheral rim of surviving cells with focal steatosis and balloon degeneration. This could be due to the formation of highly reactive radicals because of oxidative threat caused by DLA.
However, the treatment with TdPf exerted a significant inhibition of metastasis in the liver indicating their antimetastatic activity which could be comparable to that of silymarin, the standard drug used for comparison. However, TdPf was found less efficient in preventing metastasis at abdominal muscle when compared to silymarin (Plate 1c, d, h and i).

**DISCUSSION**

Many natural products have served as anticancer agents in the treatment and also as lead compounds for further research. Many plants are used to treat tumors in the Indian traditional system of medicine, but most of the plants have not been scientifically evaluated (Pushpangadan and Subramonian, 1998). Hence, enormous scope exists for identifying potent anticancer plants. Based on our earlier studies, this work was aimed to evaluate the TdPf for in vivo antitumor properties. ALT catalyses the conversion of alanine to pyruvate and glutamate and is released in a similar manner. Elevated levels of serum enzymes are indicative of cellular leakage and loss of functional integrity of cell membrane in the liver [9]. A similar significant decrease in AST, ALT, and ALP was observed by Santhi and Annapoorni [10] by the administration of silymarin to carbon tetrachloride (CCl4) induced hepatotoxicity in mice. Heba et al. [11] also reported that rats fed with powder of *Ziziphus spina-christi* fruit decrease the activities of AST, ALT, and ALP in CCl4 induced rats in a dose-dependent manner. A similar significant increase in the activity of AST in the serum of ELA induced mice was reported by Nalini et al. [12].

Elevated lipid peroxidation and a poor antioxidant system have been reported in cancer patients. Altered activities of enzymatic antioxidants are reported during carcinogenesis or after tumor formation. Hence, the elevated lipid peroxidation in the circulation of cancer animals is due to a poor antioxidant defense mechanism [13]. A decrease in SOD and CAT activities described in tumors is regarded as a marker of malignant transformation. Lowered activities of SOD and CAT were reported in several cancers [14].

Excessive generation of reactive oxygen species has been considered as hallmark in several cancers, including Dalton's ascitic tumor model [15]. Antioxidant enzymes that scavenge intermediates of oxygen reduction provide a primary defense against free radicals *in situ*. It is well known that SOD, CAT, and GPx also play important roles as protective enzymes against LPO in tissues [16]. Several investigators reported that reduced activities of SOD, CAT, and GPx in tum or-bearing animals may be due to a down-regulation of SOD and CAT genes induced by certain hormonal factors or ROS themselves (Quan et al., 2011).

Antioxidants are important substances with the ability to protect the body from damage caused by free radical-induced oxidative stress. A variety of free radical scavenging antioxidants exists within the body. Vitamin A has been associated with a decreased risk of human cancer and has protective effects in animal models of carcinogenesis. Vitamin A is known to be an important natural antioxidant capable of countering oxygen free radicals and exerting a protective antioxidant.
Santhi et al. [18] reported that Cynodon dactylon leaf protein increases the level of vitamin A in ELA induced mice. Parks and Traber [19] reported that vitamin E is one of the most important free radical scavenging chain breaking antioxidant within the biomembrane. A significant increased level of vitamin A and E in the liver and kidney of rats induced with ammonium metavanadate toxicity by the prior treatment with green tea was reported by Soussi et al. [20]. The GSH is a powerful nucleophile, critical for cellular protection nucleophile, critical for cellular protection such as detoxification from reactive oxygen species, conjugation and excretion of toxic molecules, and control of the inflammatory cytokine cascade [21]. Bigoniya and Rana [22] reported that saponin fraction of Euphorbia nerifolia has increased the levels of hepatic GSH in CCl₄ induced hepatotoxicity in rats.

CONCLUSION

This study was conducted to evaluate the antitumor activity of TdPf offered protective effect against DLA tumor by their in vivo antioxidant and antitumorigenic potential. The extract treatment at the dose of 52 µg/kg inhibited the tumor activity by the serum biochemical

| Groups          | SOD (U/mg protein)ᵃ | CAT (U/mg protein)ᵇ | GPx (U/mg protein)ᶜ |
|-----------------|---------------------|---------------------|---------------------|
|                 | 15 days             | 60 days             | 15 days             | 60 days             | 15 days             | 60 days             |
| PBS control     | 0.83±0.03           | 1.44±0.09           | 1.93±0.03           | 2.52±0.15           | 0.99±0.01           | 0.51±0.06           |
| Paraffin control| 1.85±0.03           | 0.37±0.03           | 1.94±0.02           | 2.59±0.22           | 0.93±0.04           | 1.37±0.21           |
| Silymarin       | 1.90±0.05           | 0.39±0.02           | 1.26±0.10           | 2.81±0.02           | 0.90±0.02           | 0.46±0.03           |
| TdPf            | 1.74±0.46           | 0.75±0.06           | 1.94±0.03           | 2.57±0.21           | 0.99±0.01           | 0.46±0.03           |
| TdPf+DLA        | 1.61±0.40           | 2.16±0.11           | 1.68±0.08           | 2.60±0.03           | 1.76±0.04           | 2.29±0.02           |
| DLA             | 1.61±0.40           | 2.16±0.02           | 1.68±0.08           | 2.60±0.03           | 1.76±0.04           | 2.29±0.02           |

The values mean±SD of six animals; ᵃAmount of enzyme that gives 50% inhibition of the extent of NBT reduction for SOD, ᵇamount of enzyme required to decrease the absorbance by 0.05 units at 240 nm for CAT and ᶜmicrogram of GSH utilized/minute. A significant at p<0.05 as compared to control group. SD: Standard deviation, SOD: Superoxide dismutase, CAT: Catalase, GPx: Glutathione peroxidase, DLA: Dalton’s lymphoma ascites, TdPf: Tabernaemontana divaricata

| Groups          | Vitamin A (µg/g tissue) | Vitamin E (µg/g tissue) | GSH (n moles/g tissue) |
|-----------------|------------------------|-------------------------|------------------------|
|                 | 15 days                | 60 days                 | 15 days                | 60 days                | 15 days                | 60 days                |
| PBS control     | 1.81±0.01              | 1.90±0.04               | 1.81±0.01              | 1.43±0.04              | 1.71±0.03              | 1.4±0.08               |
| Paraffin control| 1.90±0.05              | 1.43±0.01               | 1.77±0.04              | 1.70±0.04              | 1.69±0.03              | 1.74±0.04              |
| Silymarin       | 0.80±0.04              | 2.05±0.08               | 0.83±0.04              | 2.14±0.04              | 0.89±0.03              | 2.23±0.04              |
| TdPf            | 2.32±0.04              | 2.17±0.03               | 2.40±0.12              | 2.19±0.04              | 2.43±0.04              | 2.20±0.04              |
| TdPf+DLA        | 1.70±0.04              | 2.05±0.04               | 1.60±0.04              | 2.14±0.04              | 1.79±0.04              | 2.16±0.04              |
| DLA             | 1.64±0.04              | 1.57±0.04               | 1.54±0.04              | 1.70±0.04              | 1.61±0.08              | 1.74±0.04              |

The values mean±SD of six animals; A significant at p<0.05 as compared to control group. Histopathological examination. PBS: Phosphate buffered saline, DLA: Dalton’s lymphoma ascites, TdPf: Tabernaemontana divaricata

Table 1: Effect of pretreatment with crude protein extract from T. divaricata leaves on activities of enzymatic antioxidants in liver of male albino mice

Table 2: Effect of pretreatment with crude protein from T. divaricata leaves on activities of nonenzymatic antioxidants parameters in liver of male albino mice

Plate 1: (a) Normal saline, (b) paraffin oil, (c) silymarin, (d) TdPf, (e) TdPf+Dalton’s lymphoma ascites (DLA), (f), paraffin oil, (g) silymarin, (h) TdPf, (i) TdPf+DLA, (j) DLA
assessment. The extract also restored the hepatic lipid peroxidation and free radical scavenging enzyme GSH as well as other antioxidant enzymes such as SOD, CAT, and GPx in tumor-bearing mice to near normal levels. Our results suggest that whole plant extracts are promising anticancer reagents.

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