Original Article

Preliminary evaluation of *In vitro* and *In vivo* antioxidative and antitumor activities of flavonoid extract of *Tabernaemontana divaricata* leaves in Ehrlich's lymphoma and Dalton's lymphoma ascites model

ABSTRACT

Aims: In the present study, the flavonoid fraction of *Tabernaemontana divaricata* flavonoid fraction (TdFf) leaves was investigated for its *in vitro* and *in vivo* antioxidative and antitumor activity.

Subjects and Methods: The flavonoid fraction of ethyl acetate extract was assessed for their *in vitro* antioxidant activity by 2,2-diphenyl-1-picrylhydrazyl (DPPH), 2,2'-azino-bis (3-ethylbenzothiazoline-6-sulphonic acid) (ABTS), superoxide radicals, ferric reducing antioxidant power (FRAP), hydrogen peroxide, hydroxyl radicals and nitric oxide and *in vivo* antioxidative activity by enzymic and nonenzymic antioxidants in the liver of intraperitoneally implanted Ehrlich's lymphoma (EAC) and Dalton's lymphoma ascites (DLAs) model. The *in vitro* cytotoxicity was assessed using trypan blue exclusion assay and *in vivo* antitumor activity was assessed by screening the ILS, serum liver marker enzymes and histopathology of the liver.

Statistical Analysis Used: The data were expressed as the mean ± standard deviation of the means, and statistical analysis was carried out employing one-way and two-way analysis of variance using Web Agri Stat Package 2.0.

Results: The dose-dependent percentage scavenging of ABTS, DPPH, FRAP, OH, superoxide radical, and nonradical NO and H_2O_2 by TdFf indicated their antioxidative potential. Incubation of EAC/DLA tumor cells with TdFf showed a concentration-dependent cytotoxic effect, and the extract killed 50% of EAC/DLA tumor cells at a concentration of 80 μg of TdFf. Coadministration of TdFf with EAC/DLA-induced mice showed a significant increase in the liver enzymic and nonenzymic antioxidants and significant decrease in the serum liver marker enzymes to prove the *in vivo* antioxidative and antitumor activity of TdFf. It was also confirmed by the histopathology of the liver.

Conclusions: It may be concluded that the flavonoid fractions of Td possess considerable antioxidative and antitumorigenic activity against the tested DLA/EAC in both *in vitro* and *in vivo* system.

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KEY WORDS: Dalton's lymphoma ascites, Ehrlich's lymphoma ascites, flavonoid, *Tabernaemontana divaricata*

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INTRODUCTION

Cancer is a disease that is characterized by uncontrolled division of cells. These cancer cells can spread by direct growth into adjacent tissue through invasion or by implantation into distant sites by metastasis. Cancer annually affects nine million people and causes deaths around five million.[1] It is one of the most dreaded diseases of the 20th century and spreading further with the continuance of increasing incidence in the 21st century.[2] Nowadays, it is important to give more attention to identify the plant chemopreventive substances that are used to inhibit, retard, or reverse the process

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of carcinogenesis.[3] India is a vast depot of medicinal plants which are mainly used in traditional medical treatments.[4] It is reported that the natural products and related drugs are used to treat 87% of all human diseases including cancer, bacterial infection, and immune diseases.[5]

Majority of the plant active natural products are of secondary metabolites.[6] Flavonoid is one of the secondary metabolites that play an important pharmacological role against diseases such as cardiovascular disease, cancer, inflammation, and allergy.[7] Various studies have indicated that there is a relationship between flavonoid intake and reduced risk of certain cancers. In many studies of dietary prevention of cancer, flavonoids are used to inhibit cancer, particularly of breast cancer.[8] The plant, *Tabernaemontana divaricata* (Td) known as Nandhiyavattai in Tamil is an evergreen plant belonging to the family Apocynaceae. This plant is conventionally used by people in many parts of the world to treat various disorders such as abdominal tumors, arthralgia, asthma, diarrhea, epilepsy, eye infections, inflammation, leprosy, and edema.[9,10] Growing evidence suggests that this plant has medicinal benefits and its extracts could be used as a pharmacological intervention in various diseases.[11] Methanolic extract of Td exhibits the remarkable gastroprotective effect.[12] However, no study on the antitumor activity of Td has been reported. To verify and prove the antitumor activity of Td this research work was carried out.

SUBJECTS AND METHODS

Collection of plant materials and preparation of different extracts

Td leaves were collected from the various parts of Coimbatore district, Tamil Nadu. The leaves were dried and powdered. Ten grams of leaf powder was taken to prepare the aqueous, ethanol, chloroform, and ethyl acetate extracts individually.[13]

Preliminary phytochemical analysis

The condensed extracts were used for preliminary screening of phytochemicals such as carbohydrates, cholesterol, proteins, amino acids, alkaloids, saponins, flavonoids, terpenoids, cardiac glycosides, phenols, steroids, tannins, and phylobatannins.[14-17]

Preparation of flavonoid fractions of selected extracts and estimation of flavonoids

Flavonoid fractions of selected extracts were prepared by maceration.[18] Total flavonoid contents of the residue of extracts were estimated by aluminum chloride method.[19] The flavonoid fraction which showed maximum flavonoid content was selected for further studies and separated by High-performance liquid chromatography and referred as Td flavonoid fraction (TdFf).

Antioxidative potential of *Tabernaemontana divaricata* flavonoid fraction

The antioxidative potential of TdFf was evaluated by assessing the scavenging of 2,2-diphenyl-1-picrylhydrazyl (DPPH),[20] 2,2'-azino-bis(3-ethylbenzothiazoline-6-sulphonic acid) (ABTS),[21] superoxide radicals,[22] ferric reducing antioxidant power (FRAP),[23] hydrogen peroxide,[24] hydroxyl radicals,[25] and nitric oxide[26] using different concentrations.

Antitumorigenic effect (*in vitro* and *in vivo*) of *Tabernaemontana divaricata* flavonoid fraction against Ehrlich's lymphoma ascites and Dalton's lymphoma ascites tumor cells

Animals

Swiss albino mice weighing 25–30 g of either sex were used in this study. They were procured from Amala Cancer Research center, Thrissur, Kerala. The animals were acclimatized for 15 days under laboratory conditions. They were housed in polypropylene cages and maintained at 27°C ± 2°C. They were fed with standard mice feed, and water *ad libitum* was provided. The bed in the cages was changed thrice a week to maintain hygeinicity and maximum comfort for animals. Ethical clearance was obtained from the Institutional Animals Ethical Committee before the beginning of the project work for handling the animals (IAEC.2015.BC: 02).

Tumor cell lines maintenance

The tumor cell lines were procured from Amala Cancer Research Centre, Thrissur, Kerala. In mice, the tumor cells were propagated by intraperitoneal transplantation of 1×10^6 cells in 100 ml of phosphate buffered saline (PBS) after acclimatized it for 2 weeks. The bulging in the intraperitoneal cavity was seen in the mice, and the cells were drawn from the intraperitoneal cavity after 10–15 days using a syringe and used for the *in vitro* studies.

In vitro cytotoxic studies

In vitro cytotoxic studies were carried out by trypan blue exclusion method.[27] Ascitic fluid was taken from the peritoneal cavity of Ehrlich's lymphoma ascites (EAC)/Dalton's lymphoma ascites (DLA) inoculated mouse and washed with PBS. The stock cell suspension was adjusted to 1×10^6 cells by the same PBS using hemocytometer. The cells were then incubated with TdFf in a final volume of 1 ml for 3 h at room temperature. After incubation, 0.1 ml of trypan blue was added and mixed well in the incubation mixture. The total number of dead and live cells was counted using a hemocytometer, and the percentage viability/cytotoxicity was calculated. The fraction which showed the minimum concentration of flavonoid as EC_{50} was selected for the *in vivo* studies.

In vivo studies

In vivo studies were carried out by the intraperitoneal administration of EC_{50} of TdFf to assess their antitumorigenic effect. The mice were divided into eight groups with six mice in each. The study was conducted for 15, 30, 45, and 60 days. At the end of the study, the mice were sacrificed after

overnight fasting. The blood of the animals was collected by heart puncture and the serum separated was used for the estimation of liver marker enzymes. The liver was dissected, blotted of blood, and washed with PBS of pH 7.2 and used for the histological study. A portion of the liver homogenate using PBS was prepared and used for the determination of enzymic and nonenzymic antioxidants. A part of the liver homogenates using Tris HCL was prepared for the assessment of the rate of lipid peroxidation.

Antitumor effect of *Tabernaemontana divaricata* flavonoid fraction by percentage of mortality rate

In this, four groups of mice, namely, EAC, DLA-induced mice, coadministration of EAC and DLA with TdFf were used. The mortality rate of the animals due to tumor was noted, and the increase in the percentage of lifespan was calculated.[28]

Liver marker enzymes in serum

Glutamic oxaloacetic transaminase and glutamic pyruvic transaminase were assayed[29] and alkaline phosphatase.[30]

Enzymic and nonenzymic antioxidants in the liver

Enzymic antioxidants such as catalase (CAT),[31] superoxide dismutase (SOD),[32] and glutathione peroxidase (GPx)[33] were assessed in the liver. The levels of the nonenzymic antioxidants such as Vitamin A,[34] E[35] and reduced glutathione[36] were also assessed in the liver homogenate.

Lipid peroxidation in the liver

The level of TBARS was assessed[37] using the 0.1 ml of the homogenated liver.

Table 1: Phytochemical constituents of Td

Phytochemical constituents	Ethyl acetate	Chloroform	Ethanol	Aqueous
Carbohydrate	-	+	+	+
Cholesterol	+	+	-	+
Protein	+	+	-	+
Aminoacid	+	+	-	+
Alkaloid	+	+	-	+
Flavonoid	+	-	+	+
Terpenoid	-	-	-	-
Cardiac glycoside	+	+	+	+
Phenol	+	+	+	+
Glycosides	+	+	+	+
Tannin	+	+	+	+
Steroid	+	+	+	+
Saponin	-	+	+	-
Phlobatinin	+	+	-	+

Td=Tabernaemontana divaricata, (+) presence, (-) absence

Histological status of liver

The liver sample from the mice administered with TdFf and their controls were fixed in 10% formalin and then embedded in paraffin. Microtome sections of 6 μm thickness were prepared from each portion of liver and stained with hematoxylin-eosin for histopathological observation.[38]

Statistical analysis

The data were expressed as the mean ± standard deviation of the means and statistical analysis was carried out employing one-way and two-way analysis of variance using Web Agri Stat Package 2.0 (Himedia, Mumbai, Maharashtra State, India).

RESULTS

Phytochemical Screening of *Tabernaemontana divaricata* leaves

Phytochemical analysis was carried out in four different extracts, namely, aqueous, ethanol, ethyl acetate, and chloroform. It revealed the presence of various secondary metabolites such as alkaloids, flavonoids, terpenoids, glycosides, steroids, tannins, saponins, and phlobatinins as given in Table 1. However, the ethyl acetate extracts of Td was present in ethyl acetate, ethanol, and aqueous.

Total flavonoid contents of various extracts

Flavonoid was absent in chloroform extract so that the quantification was carried out only for the three leaf extracts namely ethanol, ethyl acetate, and aqueous extracts. These

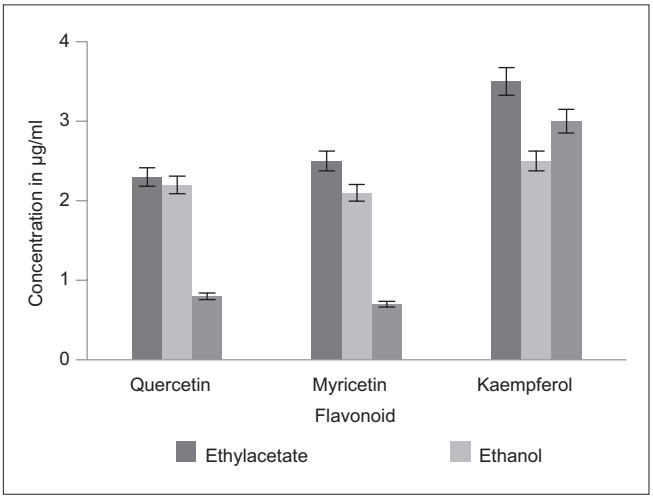

Figure 1: Flavonoid content of *Tabernaemontana divaricata*

Table 2: Average life span of DLA and EAC tumor induced mice treated with TdFf

Groups	EC50 in μg 100μl g.b.wt 1 × 106 cells	Average number of mice surviving after transplantation of tumor cells (days)						Average life span	Percentage ILS (T-C) x 100 C
		10	20	30	40	50	60		
DLA	-	6/6	4/6	0/6	0/6	0/6	0/6	20	-
EAC	-	6/6	4/6	0/6	0/6	0/6	0/6	20	-
TdFf + DLA	80 μg	6/6	6/6	6/6	5/6	5/6	4/6	46	130
TdFf + EAC	80 μg	6/6	6/6	6/6	5/6	5/6	4/6	46	130

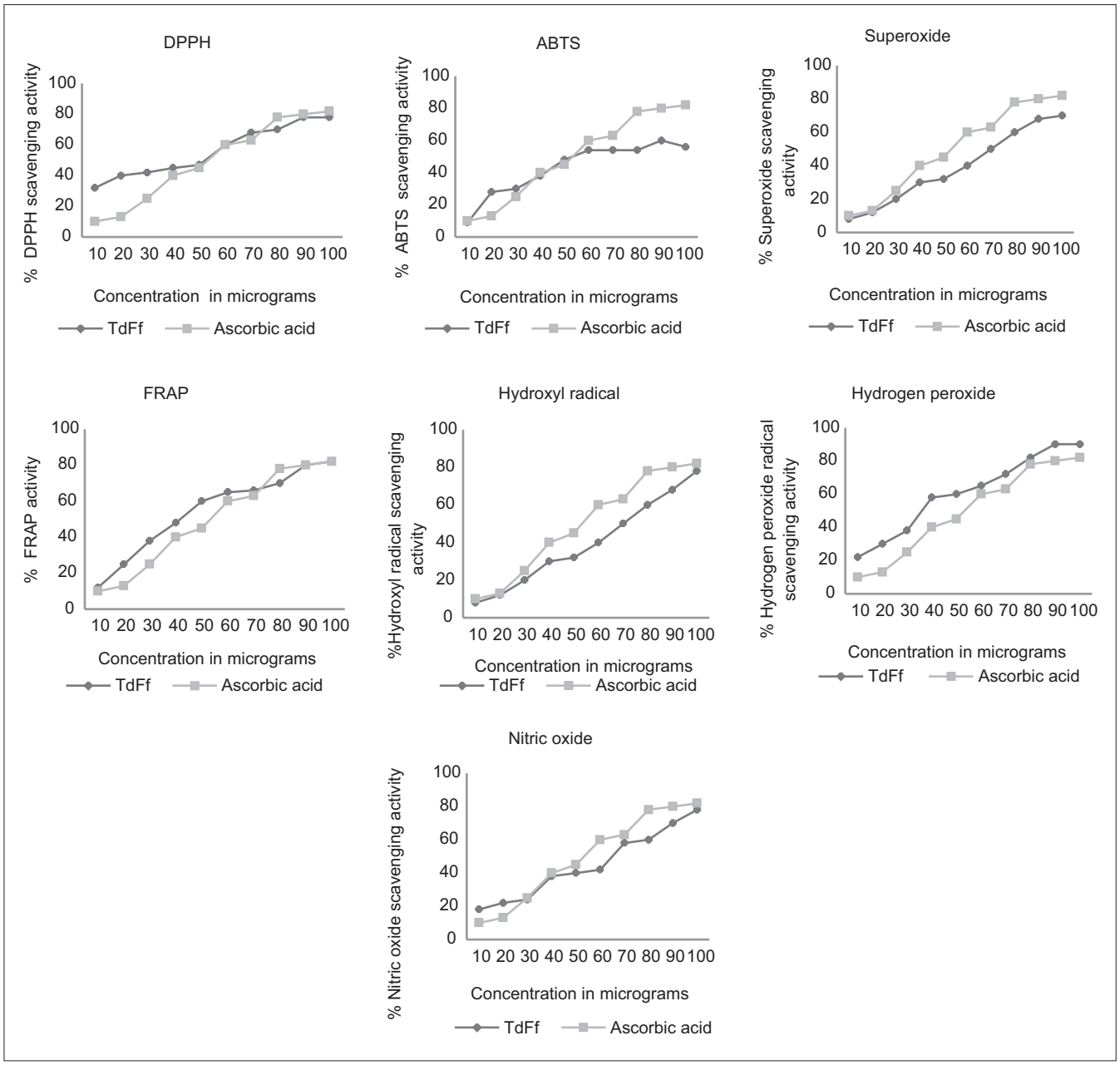

Figure 2: Percentage scavenging activity of *Tabernaemontana divaricata* flavonoid fraction

were determined against the standard flavonoids quercetin, kaempferol, and myricetin.

Figure 1 shows the flavonoid content for the three extracts. In this, the highest flavonoid content is present in ethyl acetate extract of Td leaves. Hence, further analysis of free radical scavenging activity was performed in ethyl acetate extract of Td leaves alone and was separated by HPLC which is denoted as TdFf.

Antioxidative potential of *Tabernaemontana divaricata* flavonoid fraction

The dose-dependent percentage scavenging of ABTS, DPPH, FRAP, OH, superoxide radical, and nonradical NO and H_2O_2 by TdFf indicated their antioxidative potential as shown in Figure 2. The hydroxyl radical is extremely reactive and can attack many cell constituents including lipids, nucleic acids, and proteins. The dose-dependent scavenging of the hydroxyl radical by TdFf may be due to their antioxidative role which may prevent the carcinogenic and lipid peroxidation. The dose-dependent scavenging of NO may be due to the antioxidative role of the flavonoids which may prevent all effects of excessive nitric oxide generation.

In vitro and *in vivo* antitumorigenic effect of *Tabernaemontana divaricata* flavonoid fraction against Ehrlich's lymphoma ascites and Dalton's lymphoma ascites tumor cells

In vitro cytotoxic studies

Incubation of EAC/DLA tumor cells with TdFf showed

Table 3: Non Enzymic antioxidants in the liver of control and experimental groups

Groups	Treatments	Vit A (µg/g tissue)				Vit E (µg/g tissue)				GSH (n moles/g tissue)			
		15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days	15 days	30 days	45 days	60 days
1	PBS	0.68±0.005	0.72±0.013	0.83±0.008	0.94±0.013	2.17±0.016	2.25±0.011	2.26±0.012	2.28±0.007	10.12±0.015	10.24±0.022	10.26±0.012	10.28±0.007
2	Paraffin oil	0.65±0.026	0.77±0.015	0.85±0.045	0.92±0.070	2.24±0.020	2.33±0.042	2.38±0.068	2.53±0.040	10.17±0.017	10.26±0.015	10.36±0.012	10.44±0.048
3	DMSO	0.63±0.008	0.74±0.008	0.84±0.007	0.96±0.008	2.24±0.010	2.25±0.007	2.26±0.008	2.28±0.007	10.17±0.019	10.26±0.020	10.35±0.017	10.43±0.042
4	Silymarin	0.86±0.021	1.31±0.010	1.54±0.023	2.02±0.013	3.32±0.015	3.67±0.007	3.92±0.015	4.22±0.018	11.53±0.008	11.95±0.014	12.14±0.015	12.57±0.011
6	TdFf	0.93±0.014	1.64±0.014	1.84±0.016	2.26±0.029	3.32±0.012	3.76±0.014	4.21±0.007	4.46±0.014	11.64±0.026	12.14±0.024	12.57±0.010	12.93±0.015
8	DLA + TdFf	0.83±0.027	1.17±0.022	1.44±0.009	1.95±0.017	3.14±0.009	3.77±0.015	4.02±0.013	4.11±0.020	9.16±0.008	9.74±0.016	9.93±0.020	10.14±0.071
10	EAC + TdFf	0.91±0.014	1.27±0.010	1.53±0.016	1.87±0.411	3.12±0.019	3.67±0.013	3.95±0.012	4.04±0.020	10.16±0.008	10.74±0.016	10.93±0.020	10.97±0.431
11	DLA	0.56±0.011	-	-	-	1.56±0.011	-	-	-	9.14±0.029	-	-	-
12	EAC	0.65±0.008				2.04±0.014				8.75±0.026			
#CD (0.05)		0.0183	-	-	-		0.0164	-	-	0.0201	-	-	-
##CD (0.05)		0.0194	0.0179	0.0240	0.1520	0.0167	0.0150	0.0306	0.0178	0.0167	0.0249	0.0182	0.1610
###CD (0.05)			0.0760						0.0250				0.0810

The values are the mean of six animals. #One way ANOVA (With DLA and EAC), ##One way ANOVA (Without DLA and EAC), ###Two way ANOVA

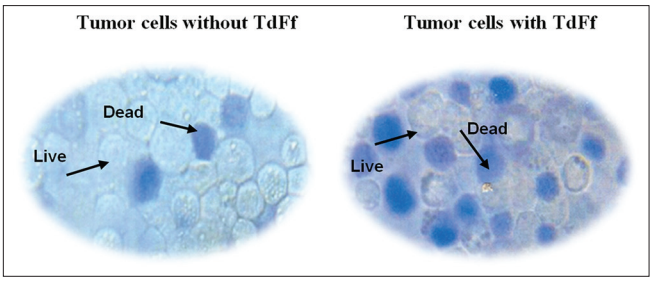

Plate 1: Antitumorigenic effect of *Tabernaemontana divaricata* flavonoid fraction to Ehrlich's lymphoma ascites/Dalton's lymphoma ascites tumor cells

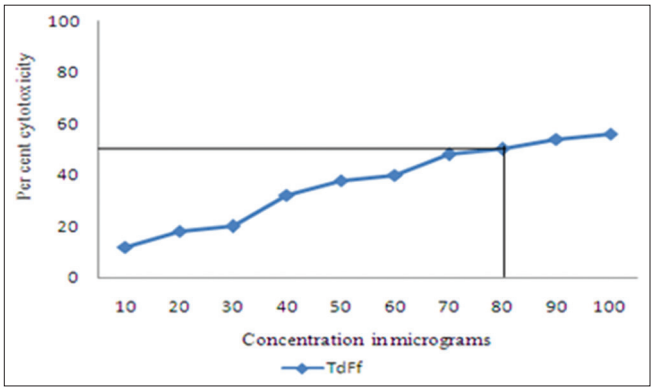

Figure 3: Cytotoxic effect of *Tabernaemontana divaricata* flavonoid fraction to Dalton's lymphoma ascites/Ehrlich's lymphoma ascites tumor cells

a concentration-dependent cytotoxic effect which was indicated by the increase in a number of dead cells with increasing concentrations of TdFf [Plate 1]. The extract killed 50% of EAC/DLA tumor cells at a concentration of 80 µg of TdFf [Figure 3]. This concentration was designated as 50% effective concentration (EC_{50}) and was used in the following *in vivo* studies.

In vivo studies

In vivo studies were carried out by the intraperitoneal administration of 80 µg (EC_{50}) of TdFf to examine their antioxidative and antitumorigenic effect.

Antitumor effect of *Tabernaemontana divaricata* flavonoid fraction by percentage of mortality rate in *in vivo* cytotoxic studies

The tumor-bearing mice lifespan was found to be 15–25 days with the average lifespan of 20 days because of tumor burden with tumor cell proliferation. Coadministration of TdFf inhibited the growth and proliferation of tumor cells, and hence, the lifespan increased which indicated their antitumorigenic effect [Table 2].

Effect of *Tabernaemontana divaricata* flavonoid fraction on the activity of liver marker enzymes in the serum of Swiss albino mice

The decrease in liver marker enzymes in the serum of mice

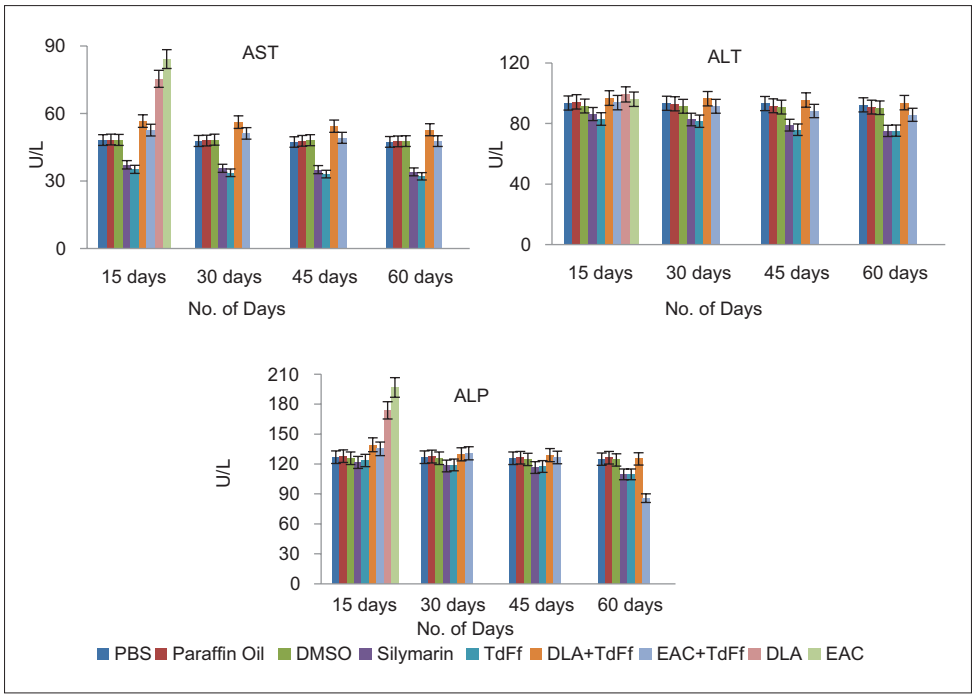

Figure 4: Liver marker enzymes in the serum of control and experimental groups

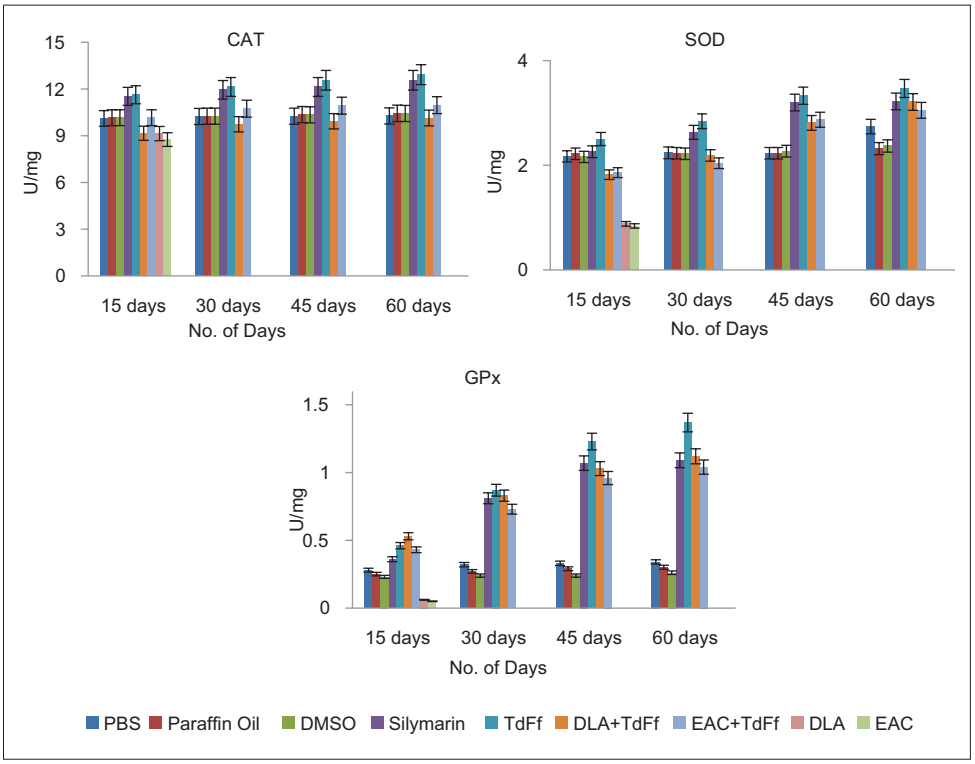

Figure 5: Enzymic antioxidants in the liver of control and experimental groups

treated with TdFf and silymarin is shown in Figure 4. The liver marker enzymes activity of TdFf administered mice showed a significant decrease on all treatment periods when compared to 15 days treatment period. Coadministration of TdFf to DLA and EAC tumor-induced mice showed a significant decrease in liver marker enzymes in all treatment periods when compared to 15 days treatment period of DLA and EAC tumor-induced mice.

DLA and EAC tumor-induced mice showed a significant increase in liver marker enzymes activity when compared to all the controls and other experimental groups in 15 days

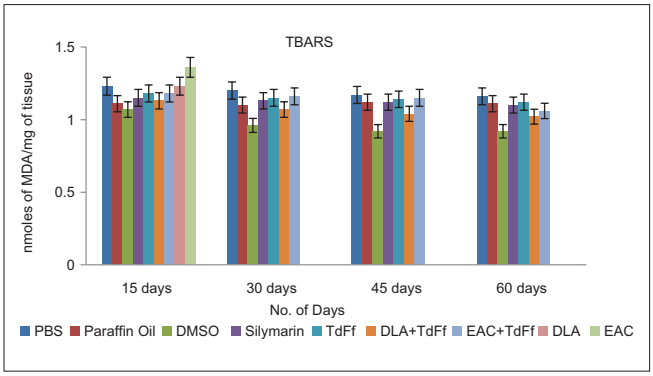

Figure 6: Levels of lipid peroxide in the liver of Swiss albino mice

treatment period. The levels of liver marker enzymes were found to be significantly increased in DLA and EAC tumor cells induced mice and indicated the membrane damage, whereas TdFf treatment in tumor cells induced mice showed a significant decrease in the liver marker enzymes activity and indicated their protective role against DLA and EAC tumor-induced toxicity.

Effect of *Tabernaemontana divaricata* flavonoid fraction on the activities enzymic and nonenzymic antioxidants in the liver of swiss albino mice

The enzymic and nonenzymic antioxidants activities were found to be significantly increased in the mice administered with TdFf when compared to PBS control mice in 15, 30, 45, and 60 days treatment periods. Compared to standard antioxidant silymarin, TdFf administration showed a significant increase in the enzyme activities on all the experimental periods. Coadministration of TdFf in DLA and EAC induced mice also showed significant enhanced activity in all the treatment periods when compared DLA and EAC induced mice. As the treatment period increased, the activity of them was also found to be increased as presented in Figure 5 and Table 3.

The administration of TdFf exhibited a significant increase in the levels of all the nonenzymic antioxidants, and it clearly indicated that TdFf is a very good inducer of enzymic and nonenzymic antioxidants.

Effect of *Tabernaemontana divaricata* flavonoid fraction on lipid peroxidation in the liver of Swiss albino mice

The level of lipid peroxides in the liver was found to be significantly decreased in all the treatment periods by the administration of TdFf when compared to PBS control. The level of lipid peroxides in the standard antioxidant treated mice was found to be significantly decreased when compared to their control mice on all treatment periods. The mice transplanted with DLA and EAC tumor cells showed a significant increase in lipid peroxides when compared to the controls and experimental groups in 15 days treatment period. Coadministration of TdFf to DLA and EAC tumor-induced mice showed significantly decreased levels of lipid peroxide in the liver when compared to the tumor-induced mice on 15 days treatment period. Both flavonoid fractions administration showed more significantly decreased levels of lipid peroxide in all treatment periods than that of silymarin administered mice as shown in Figure 6.

Effect of *Tabernaemontana divaricata* flavonoid fraction on the histological status of the liver in Dalton's lymphoma ascites and Ehrlich's lymphoma ascites challenged mice

Histological examination of tissues indicated the malignant changes observed in the DLA and EAC tumor-induced liver sections as shown in Plate 2. PBS: Liver parenchyma with normal central vein and blood vessels. Paraffin Oil: Hepatic cells nuclei were found to be normal. DMSO: the Normal architecture of portal triads, sinusoids, Kupffer cells and central vein. Silymarin: Normal with congestion of blood vessel. The occasional tract contains mononuclear inflammatory cells. Swollen hepatocytes and prominent Kupffer cells were observed in the lobules. TdFf: Portal triad and central vein were found to be normal with swollen hepatocytes and some with enlarged nuclei in the lobular region. TdFf + DLA: The portal triads, parenchyma with nuclei of hepatic cells were found to be normal with marked sinusoidal and central vein congestion. TdFf + EAC: The portal triads, parenchyma with nuclei of hepatic cells were found to be normal with marked sinusoidal and central vein congestion. DLA and EAC: Dilated central vein was found. The portal triad showed a collection of lymphocytes, slight enlargement and macrovesicular fatty changes in the hepatocytes, dilated sinusoidal spaces containing lymphocytes, vacuoles filled hepatocytes with nuclear changes including necrosis.

DISCUSSION

The presence of many important secondary metabolites indicates that the plant possesses high profile values and can be used to treat various diseases. It gives important information about the different phytoconstituents present in the extracts to help the future investigators regarding the selection of the particular extract for further investigation of isolating the active principle.[39] Flavonoids are one of the varied and prevalent groups of natural compounds that are probably the most important natural phenolics. They are very key constituents of plants because of the scavenging aptitude conferred by their hydroxyl groups. They may contribute directly to antioxidative action. In humans, daily consumption of flavonoid-rich fruits and vegetables inhibits mutagenesis and carcinogenesis.[40]

DPPH is one of the few stable and commercially accessible organic nitrogen radicals.[41] A stable ABTS radical cation, which has blue–green chromophore absorption, was produced by oxidation of ABTS with potassium persulfate before the addition of antioxidants.[42] The antioxidant activity of the natural products is determined by the decolorization of the ABTS, by measuring the absorbance at 734 nm.[43] The hydroxyl radical scavenging assay allows assessment of abilities of

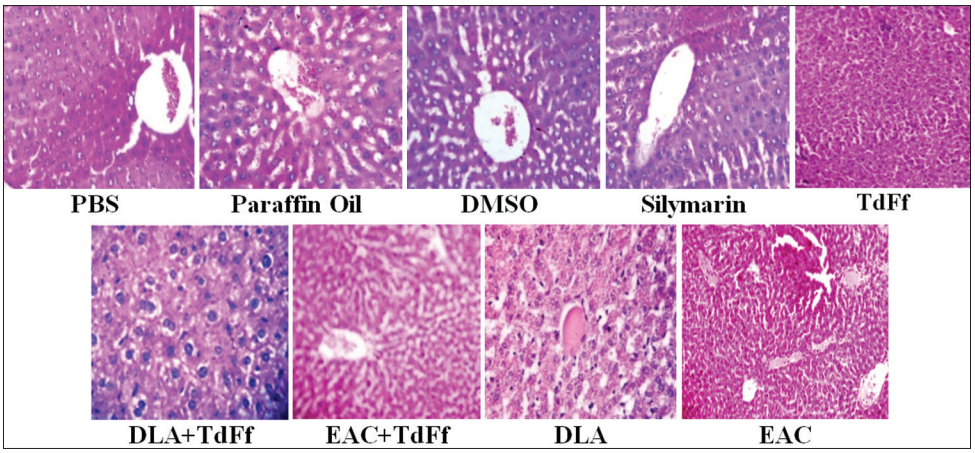

Plate 2: Histological status of the liver

extracts to exert pro-oxidant action, scavenge hydroxyl radicals generated by Fenton systems, and assessment of abilities to chelate metal iron.[44] FRAP assay measures directly the ability of antioxidants to reduce a ferric tripyridyltriazine complex (Fe^{3+}-TPTZ) to the ferrous complex (Fe^{2+}-TPTZ).[45] The antioxidative efficacy may be due to the flavonoids of the ethyl acetate extracts of Td.

Cytotoxicity is based on the principle that live cells possess intact cell membrane that excludes the dye while the dead cells do not and have blue colored cytoplasm under light microscope.[46] The study was supported by researchers who showed maximum cell cytotoxicity effect to DLA tumor cells of four ayurvedic herbs such as *Curcuma longa L., Ocimum sanctum L., Tinospora cordifolia (Wild) and Zizyphus mauritiana.*[47] An author revealed the potent antitumorigenic effect of aqueous extract of *Areca catechu.*[48] Similar work also reported the cytotoxic effect of methanolic extracts of stem and rhizomes of *Berberis aristata* and *Hemidesmus indicus* in MCF-7 breast cancer cell line.[49]

By the administration of the methanolic extract of *Plumeria alba* leaves, there is an increase in the lifespan of DLA and EAC against Swiss albino mice.[50] Similar antitumor properties of *Jasminum sambac* (*Linn*) was investigated *in vivo* and *in vitro* using Dalton's ascites lymphoma model[51] and methanolic extract of *Argemone mexicana* Linn against the HeLa and MCF-7 cells.

In one study, an author reported that the methanolic extract of *Costus pictus* showed a significant decrease in the ALP, AST and ALT levels in the serum of rats.[52] In a study, they also investigated the similar significant decrease in the activity of AST, ALT, and ALP by the leaf extract of *Cordia macleodii* treatment in the rat.[53] The activity of ALT was significant decreases in the serum of EAC tumor burden and by the coadministration of protein fraction to EAC tumor-induced mice.[54,55]

The methanolic fraction of *Jatropha curcas* was found to normalize the decreased activities of SOD, CAT, GPx, GST, and GR in hepatic carcinoma induced rats.[56] Administration of *Cynodon dactylon* leaf protein also enhanced the levels of nonenzymic antioxidants (Vitamin A, Vitamin E, and GSH) in EAC-induced mice.[57]

The methanolic extract of the plant *Hedyotis corymbosa* significantly reduced the accumulation of lipid peroxides in rat liver homogenate.[58] Researchers found decreased levels of TBARS in serum, liver and in kidney in DLA tumor-induced mice treated with a methanolic extract of the bark of *Careya arborea Roxb.*[59] The methanolic extract of the whole plant of *Amaranthus spinosus* showed potent hepatoprotective activity against carbon tetrachloride induced hepatic damage in rats and significantly altered malondialdehyde levels.[60]

Histopathological examination of heart, kidney, and liver of the low-dose and mid-dose drug-treated groups showed no significant changes in the cytoarchitecture of any organs studied in both the kidney and liver.[61] It may be concluded that the flavonoid fractions of Td exhibited EC_{50} value of 80 µg in 100 µl of PBS that possesses considerable antioxidative and antitumorigenic activity against the tested DLA/EAC in both *in vitro* and *in vivo* system which offers a future study for the development of drug designing and also justifies the medicinal values of the plants.

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Conflicts of interest

There are no conflicts of interest.

REFERENCES

1. Chaudhuri P, Maji T, Ray KD. Cancer and radiotherapy. Sci Cult 2004;70:142-5.

2. Balachandran P, Govindarajan R. Cancer – An ayurvedic perspective. Pharmacol Res 2005;51:19-30.
3. Shukla Y, Kalra N. Cancer chemoprevention with garlic and its constituents. Cancer Lett 2007;247:167-81.
4. Ballabh B, Chaurasia OP. Traditional medicinal plants of cold desert ladakh – Used in treatment of cold, cough and fever. J Ethnopharmacol 2007;112:341-9.
5. Chin YW, Balunas MJ, Chai HB, Kinghorn AD. Drug discovery from natural sources. AAPS J 2006;8:E239-53.
6. Mulabagal V, Chen-Yue L, Shu-Fung L, Satish MN, Chien YL, Hsin-Sheng T. Studies on the production of some important secondary metabolites from medicinal plants by tissue cultures. Bot Bull Acad Sin 2004;45:1-22.
7. Yao LH, Jiang YM, Shi J, Tomás-Barberán FA, Datta N, Singanusong R, *et al.* Flavonoids in food and their health benefits. Plant Foods Hum Nutr 2004;59:113-22.
8. Wang Y, Chan FL, Chen S, Leung LK. The plant polyphenol butein inhibits testosterone-induced proliferation in breast cancer cells expressing aromatase. Life Sci 2005;77:39-51.
9. Warrier PK, Nambiar VP, Ramankutty C. Indian Medicinal Plants. Vol. 2. Madras: Orient Longman Ltd.; 1996. p. 232.
10. Kirthikar KR, Basu BD. Indian Medicinal Plants. Vol. 3. Dehradun: New India Publishing Agency; 1998. p. 577-8.
11. Pratchayasakul W, Pongchaidecha A, Chattipakorn N, Chattipakorn S. Ethnobotany & ethnopharmacology of *Tabernaemontana divaricata*. Indian J Med Res 2008;127:317-35.
12. Ali Khan MS. Gastoprotective effect of *Tabernaemontana divaricata* (Linn) R.Br. Flower methanolic extract in wistar rats. Br J Pharm Res 2011;1:88-98.
13. Santhi R, Lakshmi G, Priyadharshini AM, Anandaraj L. Phytochemical screening of *Nerium oleander* leaves and *Momordica charantia* leaves. Int Res J Pharm 2011;2:131-5.
14. Kumar G, Karthik L, Rao KV. Phytochemical composition and *in vitro* antimicrobial activity of *Bauhinia racemosa* lamk (caesalpiniaceae). Int J Pharm Sci Res 2010;1:51-8.
15. Ayoola GA, Sofidiya T, Odukoya O, Coker HA. Phytochemical screening and free radical scavenging activity of some Nigerian medicinal plants. J Pharm Sci Pharm Pract 2006;8:133-6.
16. Edeoga HO, Gomina A. Nutritional values of some nonconventional leafy vegetables of Nigeria. J Econ Taxon Bot 2000;24:7-13.
17. Raaman N. Phytochemical Techniques. New Delhi: New India Publishing Agency; 2006.
18. Solank R, Nagori BP. New method for extracting phytoconstituents from plants. International Journal of Biomedical and Advanced Research 2012;03:770-4.
19. Chang CC, Yang MH, Wen HM, Chern JC. Estimation of total flavonoid content in propolis by two complementary colorimetric methods. J Food Drug Anal 2002;10:178-82.
20. Mensor LL, Menezes FS, Leitão GG, Reis AS, dos Santos TC, Coube CS, *et al.* Screening of Brazilian plant extracts for antioxidant activity by the use of DPPH free radical method. Phytother Res 2001;15:127-30.
21. Shirwaikar A, Devi S, Premalatha K, Siju EN. *In vitro* anthelmintic activity of *Thespesia populnea*. Asian J Biochem Pharm Res 2011;3:5.
22. Winterbourn CC, Hawkins RE, Brian M, Carrell RW. The estimation of red cell superoxide dismutase activity. J Lab Clin Med 1975;85:337-41.
23. Benzie IF, Strain JJ. Ferric reducing/antioxidant power assay: Direct measure of total antioxidant activity of biological fluids and modified version for simultaneous measurement of total antioxidant power and ascorbic acid concentration. Methods Enzymol 1999;299:15-27.
24. Ruch RJ, Cheng SJ, Klaunig JE. Prevention of cytotoxicity and inhibition of intercellular communication by antioxidant catechins isolated from Chinese green tea. Carcinogenesis 1989;10:1003-8.
25. Elizabeth K, Rao MW. Oxygen radical scavenging activity of curcumin. Int J Pham 1990;58:237-40.
26. Green LC, Wagner DA, Glogowski J, Skipper PL, Wishnok JS, Tannenbaum SR, *et al.* Analysis of nitrate, nitrite, and [15N] nitrate in biological fluids. Anal Biochem 1982;126:131-8.
27. Salomi MJ, Panikkar KR. Cytotoxic action of *Nigella sativa* seeds. Amala Res Bull 1989;11:60-3.
28. Geran RI, Greenberg HM, McDonald M, Abbott BJ. Protocols for screening chemical agents and natural products against animal tumors and other biological systems. Cancer Chemoth Rep 1972;33:1-17.
29. Reitman S, Frankel S. A colorimetric method for the determination of serum glutamic oxalacetic and glutamic pyruvic transaminases. Am J Clin Pathol 1957;28:56-63.
30. King J. The hydrolases-acid and alkaline phosphatases. In: Van D, editor. Practical Clinical Enzymology. J C King: Nostrand Company; 1965. p. 363-95.
31. Luck H. In: Methods in Enzymatic Analysis. 2nd ed. New York: Bergmeyer; 1974. p. 885.
32. Misra HP, Fridovich I. The role of superoxide anion in the autoxidation of epinephrine and a simple assay for superoxide dismutase. J Biol Chem 1972;247:3170-5.
33. Rotruck JT, Pope AL, Ganther HE, Swanson AB, Hafeman DG, Hoekstra WG, *et al.* Selenium: Biochemical role as a component of glutathione peroxidase. Science 1973;179:588-90.
34. Bayfield RF, Cole ER. Colorimetric estimation of Vitamin A with trichloroacetic acid. In: McCormick DB, Wright LD, editors. Enzymol. Part F, Vitamins and Coenzymes. Vol. 67. New York: Academic Press; 1980. p. 189-95.
35. Rosenberg HR. Chemistry and Physiology of the Vitamins. New York: Interscience Publishers; 1992. p. 452-3.
36. Moron MS, Depierre JW, Mannervik B. Levels of glutathione, glutathione reductase and glutathione S-transferase activities in rat lung and liver. Biochim Biophys Acta 1979;582:67-78.
37. Niehaus WG Jr., Samuelsson B. Formation of malonaldehyde from phospholipid arachidonate during microsomal lipid peroxidation. Eur J Biochem 1968;6:126-30.
38. Culling CF. Handbook of Histopathology and Hitochemistry Techniques. 3rd ed. London: Butterworths and Co Ltd.; 1974. p. 115-7.
39. Mishra SB, Mukerjee A, Vijayakumar M. Pharmacognostical and phytochemical evaluation of leaves extract of *Jatropha curcas* linn. Pharm J 2010;2:9-14.
40. Tanaka M, Kuei CW, Nagashima Y, Taguchi T. Application of antioxidative maillrad reaction products from histidine and glucose to sardine products. Bull Jpn Soc Sci Fish 1988;54:1409-14.
41. MacDonald-Wicks LK, Wood LG, Garg ML. Methodology for the determination of biological antioxidant capacity *in vitro*: A review. J Sci Food Agric 2006;86:2046-56.
42. Stratil P, Klejdus B, Kubán V. Determination of total content of phenolic compounds and their antioxidant activity in vegetables – Evaluation of spectrophotometric methods. J Agric Food Chem 2006;54:607-16.
43. Biglari F, AlKarkhi AF, Easa AM. Antioxidant activity and phenolic content of various date palm (*Phoenix dactylifera*) fruits from Iran. Food Chem 2008;107:1636-41.
44. Aruoma OI. Methodological considerations for characterizing potential antioxidant actions of bioactive components in plant foods. Mutat Res 2003;523-524:9-20.
45. Alanon ME, Castro-Vazquez L, Diaz-Maroto MC, Hermosin-Gutierrez I, Gordon MH, Perez-Coello MS. Antioxidant capacity and phenolic composition of different woods used in cooperage. Food Chem 2011;129:1584-90.
46. Jayaseelan RS, Vijayan FP, Mathesvaran M, Suresh V, Padikkala J. Cytotoxic and antitumor activity of methonolic extracts *Desmodium* triangulare (Merr) Root. Int J Pharm Sci 2012;4:540-2.
47. Adhvaryu MR, Reddy N, Parabia MH. Anti-tumor activity of four ayurvedic herbs in dalton lymphoma ascites bearing mice and their short-term *in vitro* cytotoxicity on DLA-cell-line. Afr J Tradit Complement Altern Med 2008;5:409-18.
48. Anajwala CC, Patel RM, Dakhara SL, Jariwala JK. *In vitro* cytotoxicity study of agave Americana, *Strychnos nuxvomica* and *Areca catechu*

extracts using mcf-7 cell line. J Adv Pharm Technol Res 2010;1:245-52.
49. Mazumder PM, Saumya D, Das MK. Cytotoxic activity of methanolic extracts of *Berberis aristata* and *Hemidesmus indicus* R.Br.in MCF7 cell line. J Curr Pharm Res 2010;112-5.
50. Radha R, Sivakumar T. *In vitro*, *in vivo* anticancer activity of leaves of *Plumeria alba* (Linn). J Pharm Res 2009;2:203-7.
51. Kalaiselvi M, Narmadha R, Ragavendran P, Ravikumar G, Gomathi D, Sophia D, *et al*. *In vivo* and *in vitro* antitumor activity of *Jasminum sambac* (*Linn) oleaceae* flower against daltons ascites lymphoma induced swiss albino mice. Int J Pharm Sci 2012;4:145-7.
52. Jothivel N, Ponnusamy SP, Appachi M, Singaravel S, Radilingam D, Deivasigammi K, *et al*. Antidiabetic activity of methanol leaf extract of *Costus pictus* don in alloxan induced diabetic rats. J Health Sci 2007;53:655-63.
53. Qureshi SA, Asad W, Sultana V. The effect of *Phyllantus emblica* linn on type – II diabetes, triglycerides and liver – Specific enzyme. Pak J Nutr 2009;8:125-8.
54. Santhi R, Annapoorani S. Efficacy of *Cynodon dactylon* for immunomodulatory activity. Drug Invent Today 2010;2:112-4.
55. Chandra Prabha D, Annapoorani S. Antioxidant status in the mice treated with the protein fraction of the grass *Cynodon dactylon*. J Ecotoxi Environ Monit 2011;20:501-8.
56. Balaji R, Suba V, Rekha N, Deecaraman M. Hepatoprotective activity of methanolic fraction of *Jatropha curcas* on afflatoxin B1 induced hepatic carcinoma. Int J Pharm Sci 2009;1:287-96.
57. Santhi R, Kalaiselvi K, Annapoorani S. Antioxidant efficacy of *Cynodon dactylon* leaf protein against ELA implanted Swiss albino mice. J Pharm Res 2010;3:228-30.
58. Sadasivan S, Latha PG, Sasikumar JM, Rajashekaran S, Shyamal S, Shine VJ, *et al.* Hepatoprotective studies on *Hedyotis corymbosa* (L.) lam. J Ethnopharmacol 2006;106:245-9.
59. Natesan S, Badami S, Dongre SH, Godavarthi A. Antitumor activity and antioxidant status of the methanol extract of *Careya arborea* bark against dalton's lymphoma ascites-induced ascitic and solid tumor in mice. J Pharmacol Sci 2007;103:12-23.
60. Zeashan H, Amresh G, Singh S, Rao CV. Hepatoprotective activity of *Amaranthus spinosus* in experimental animals. Food Chem Toxicol 2008;46:3417-21.
61. Simna SP, Umadevi S, Gopi V, Elangovan V. Toxicological assessment of herbomineral siddha formulation: Neraadimuthuvallathy mezhugu in swiss albino mice. J Pharm Res 2012;5:1684-9.