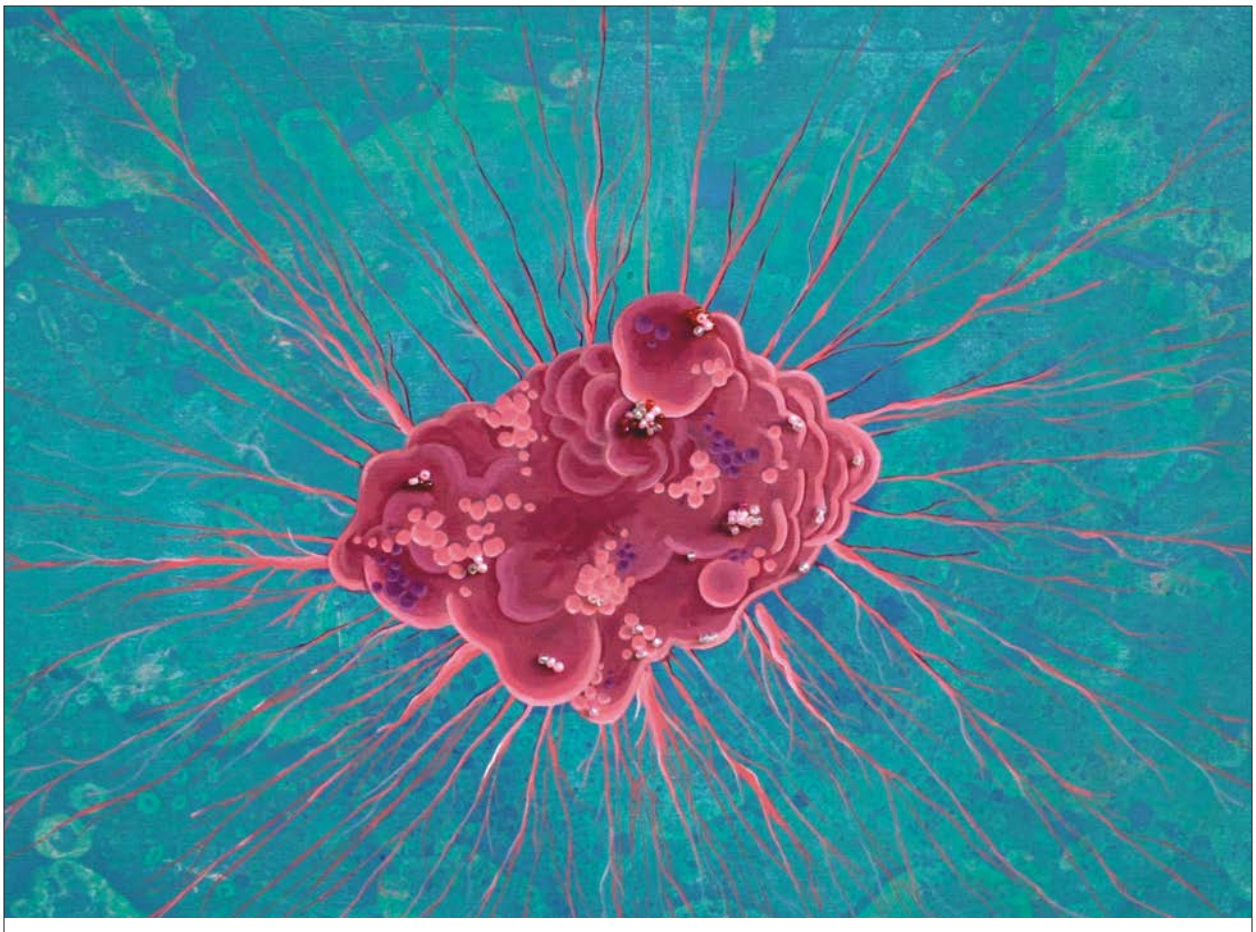

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Role of Glutathione and Glutathione-Related Enzymes in the Antitumor Activity of *Dillenia pentagyna* in Dalton's Lymphoma-Bearing Mice

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Abstract: The aim of present study was to determine the antitumor potential of *D. pentagyna* and the level of glutathione and glutathione-related enzyme activities (i.e., glutathione-s-transferase, glutathione reductase and glutathione peroxidase) in the host tissues in order to find their possible involvement in the antitumor activity of *D. pentagyna*. The most potent antitumor activity of methanol extract of *D. pentagyna* (%ILS~70) was observed at a dose of 20 mg kg^{-1} body wt. day^{-1}. The present finding shows a significant *D. pentagyna* extract-mediated change in the level of GSH and GSH-related enzyme activities in different tissues at different interval of time. A significant decrease in the level of GSH, glutathione reductase and glutathione peroxidase activities were noted in DL cells after plant extract treatment (20 mg kg^{-1} body wt.). These findings suggest that the plant extract-mediated decrease of GSH, GR and GPx in DL cells may have a role in the antitumor activity of *D. pentagyna* by decreasing the protective ability of DL cells, thereby enhancing tumor cell death and increasing host survivability.

Key words: *Dillenia pentagyna,* glutathione, glutathione s-transferase, glutathione reductase, glutathione peroxidase, Dalton's lymphoma

INTRODUCTION

Our preliminary investigation through literature search, personal interview with elders and local herbal practitioners from Mizoram state, India, revealed the use of *Dillenia pentagyna* Roxb. (*D. pentagyna*) (Dilleniaceae) as a traditional medicine for the treatment of cancer suspected diseases and other stomach ailments. For the treatment of cancer and other stomach ailments, the local people (Mizos) of this state prepared the plant medicine by grinding the stem bark and boiling the ground tissue powder with water for 15 to 20 min and 100 to 200 mL of the juice collected was taken twice a day after food. However, there is no scientific scrutiny about the antitumor potential of *D. pentagyna*.

Reduced glutathione (a tripeptide, L-γ-glutamyl-L-cysteinyl-glycine, GSH) is an important cellular antioxidant responsible for many functions including maintenance of protein structure and function by reducing the disulphide linkages of proteins, regulation of protein synthesis and degradation, maintenance of enzyme activity and immune function, protection against oxidative damage, detoxification of xenobiotics and in drug metabolism (Wang and Ballatori, 1998). GSH and its related enzymes work with other antioxidants and antioxidant enzymes to protect cells from oxidative damage caused by Reactive Oxygen Intermediates (ROIs) (Zubkova and Robaire, 2004). Elevation of intracellular GSH levels has also been suggested to be involved in the resistance of cancer cells to oxidative stress, radiotherapy and chemotherapy (Navarro *et al*., 1999), while a depletion of GSH

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levels could increase the cytotoxicity of a variety of antitumor agents (Khynriam and Prasad, 2003) which in turn could induce the apoptotic cell death also (Kane *et al*., 1993). Therefore, changes in the rate of cancer cell proliferation are accompanied by changes in their intracellular GSH levels and consequently, these could be reflected by changes in the antioxidant machinery. Glutathione-related antioxidant enzymes have been reported to be involved in the detoxification of peroxides, xenobiotics, hydroperoxides and drugs (Chasseaud, 1979; Meyer *et al*., 1998) and some of the excess oxygen radicals such as hydrogen peroxide, superoxides, hydroperoxy and hydroxyl radicals are shown to be implicated in a variety of disorders including cardiovascular disease, rheumatoid arthritis, immune injury and cancer (Ross, 1988). In the detoxification of these reactive free radicals, some of the GSH-related enzymes such as glutathione-s-transferase (GST), glutathione reductase (GR) and glutathione peroxidise (GPx) are involved in the intracellular defence mechanisms (Tew, 1994; Noctor and Foyer, 1998; Teramoto *et al*., 1999; Ohkuwa *et al*., 1997). Therefore, treatment strategies involving GSH depletion and alteration of GSH related enzymes may also be taken into consideration in order to maximize the therapeutic efficacy of anticancer agents.

All these information generated an interest to investigate the antitumor potential of *D. pentagyna* in Dalton's lymphoma-bearing mice and to determine the level of glutathione and some glutathione-related enzyme activities in various tissues of Dalton's lymphoma-bearing mice under different treatment conditions in order to find their possible involvement in the antitumor activity of *D. pentagyna*.

MATERIALS AND METHODS

Materials

The stem bark of *D. pentagyna* was collected from Kawlkulh village, Mizoram state, India on April 2007 and the methanol extract was prepared as described previously (Rosangkima and Prasad, 2004). Briefly, freshly collected plant tissue was shade-dried in an oven under 40-45°C. The dried material was grounded using sterilized mortar and pestle. Five hundred gram of powdered material was extracted with 2 L of absolute methanol at room temperature for 24 h. The tissue-solvent mixture was filtered using Whitman No. 1 filter paper and the filtrate was evaporated to dryness in a rotary evaporator. The dry mass obtained was collected and stored under 0°C until used. Methanol extract was tried to dissolve preferentially in double distilled water, phosphate-buffered saline (PBS, pH 7.4), methanol and sodium hydroxide solutions. 0.05% NaOH (minimum concentration showing maximum solubility of the extract) was selected to dissolve the plant extract for the experiment. Reduced glutathione, oxidized glutathione (GSSG), glutathione reductase (GR), [5, 5'-dithiobis-(2-nitrobenzoic acid)] (DTNB) and CDNB were purchased from Sigma Chemical Company St. Louis, MO, USA. Nicotinamide adenine dinucleotide phosphate reduced (NADPH), 1-chloro-2,4-dinitrobenzene (CDNB), ethylenediaminetetra-acetic acid (EDTA) and other chemicals used in the experiments were of analytical grade and purchased from SRL Pvt. Ltd., Mumbai, India.

Animals and Tumor Maintenance

Inbred Swiss albino mice (male) in the age group of about 10-12 weeks old were used for the experiments. All mice were maintained in the laboratory under conventional conditions at room temperature of 20±2°C with free access to food pellets (Amrut Laboratory, New Delhi) and water *ad libitum*. Ascites Dalton's lymphoma is being maintained in mice by serial intraperitoneal (i.p.) transplantations of approximately 1×10^7 viable tumor cells per animal [0.25 mL in phosphate-buffered saline (PBS), pH 7.4]. Early sign of tumor development was visible after 3 to 4 days of tumor transplantation. Tumor transplanted hosts usually survived for 19-21 days.

Antitumor Study

Antitumor activity of methanol extract of *D. pentagyna* was studied following the method of Sakagami *et al*. (1987). 1×10^7 viable tumor cells were transplanted intraperitoneally in 10-12 weeks old male mice (20-23 g body wt.). The day of tumor transplantation was designed as day 0. Plant extract treatment was given for 5 consecutive days starting from day 1 of tumor transplantation and the host survival patterns were recorded. Different doses of plant extract (10 to 200 mg kg^{-1} body wt. day^{-1}) was used for the study and the antitumor efficacy was reported in percentage of average increase in life span (ILS) calculated using the formula (T/C×100)-100, where, T and C are the mean survival days of treated and control groups of mice, respectively. Control and different groups of mice with different doses consisted of 10 mice each. The most potent dose (20 mg kg^{-1} body wt.) was selected for further enzymatic studies.

Treatment

In glutathione and enzymatic studies, animals were divided into four groups. Group 1 consisted of normal mice without tumor. Group 2 consisted of tumor-bearing mice. Group 3 consisted of tumor-bearing control receiving 0.25 mL of plant extract vehicle (0.05% NaOH) and Group 4 consisted of tumor-bearing mice receiving a single intraperitoneal (i.p) injection of *Dillenia pentagyna* extract (DPE, 20 mg kg^{-1} body wt.) on the 10th day of post-tumor transplantation which is the logarithmic phase of tumor growth.

Changes in Body Weight and Food Consumption

Changes in the average body weight and food consumption of animals in different groups (1, 3 and 4) consisting of 6 animals in each group, were monitored for 18 days from the day of tumor transplantation. The results are expressed as Mean±SD.

Determination of Glutathione and Enzyme Activities

In order to study the pattern of changes in glutathione and enzyme activities during tumor growth, group-2 animals were killed by cervical dislocation on the 5 and 10th day of tumor growth, collected tissues and used for the study. After 24, 48, 72 and 96 h of treatment, animals from group-3 (control) and 4 (DPE treated) were killed by cervical dislocation and different tissues (liver, kidney, spleen, testes and DL cells) were collected and used for determination of glutathione and enzymatic activities. Glutathione concentrations were determined using the method described by Sedlak and Lindsay (1968). Glutathione-s-transferase activity GST (Habig *et al*., 1974), glutathione reductase activity GR (Smith *et al*., 1988) and glutathione peroxidase activity Gpx (Flohe and Gunzler, 1984) were measured by the methods indicated in parentheses. Protein concentrations were also measured according to the method of Lowry *et al*. (1951).

Statistical Analysis

All values reported in this study are Mean±SD. Significant differences between the control and different treatments were calculated using Student's t-test. Number of replicates (N) = 6. p-values of ≤ 0.05 were considered significant. The significance of the total changes between control and treated groups was also tested by one way ANOVA.

RESULTS

Methanol extract of stem bark of *D. pentagyna* dose-dependently increased the survivability of tumor-bearing mice. Among different doses used, 20 mg kg^{-1} body wt. day^{-1} showed comparatively better antitumor activity (%ILS~70) against ascites Dalton's lymphoma (Fig. 1).

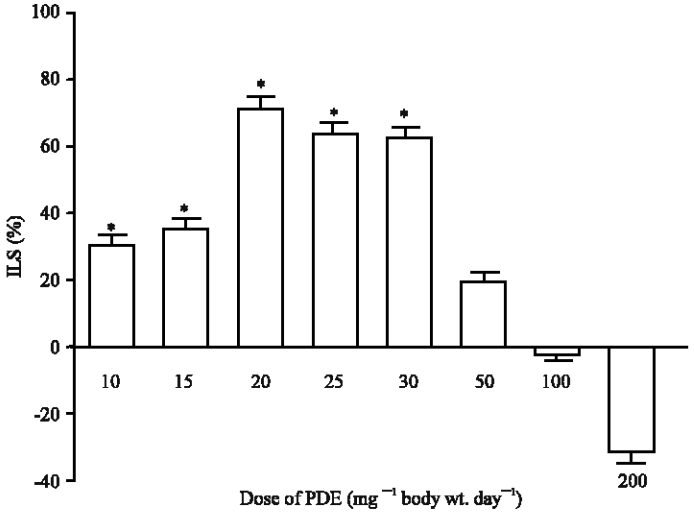

Fig. 1: Graph showing percentage increase in life span (ILS%) of tumor-bearing mice treated with different doses of DPE. Results are Mean±SD, *Doses of DPE showing ILS% = 20 were considered to possess antitumor potential

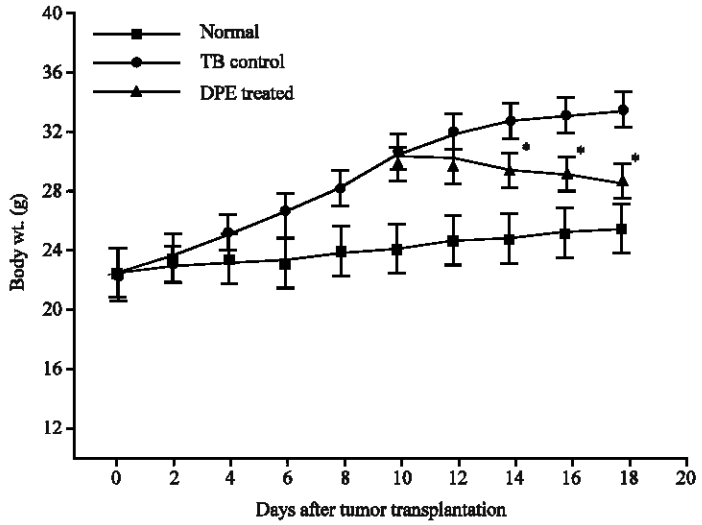

Fig. 2: Graph showing changes in the body weight of normal, tumor-bearing control and DPE treated mice (20 mg kg^{-1} body wt.). The results are expressed as Mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control, *p≤0.05

During tumor growth progression, there was a rapid increase in the body weight of control tumor-bearing mice reaching 33 g on the 18th day of tumor growth. DPE treatment on the 10th day at a dose of 20 mg kg^{-1} body wt. significantly decreased the body weight of tumor-bearing mice after 14th day of tumor growth (Fig. 2). The average food consumption of normal mice is 7.5 g. In control tumor-bearing mice, a gradual decrease in the food consumption was noted. DPE treatment (20 mg kg^{-1} body wt.) on the 10th day of tumor growth significantly increased the total food consumption of tumor-bearing mice after 14th day of tumor growth (Fig. 3).

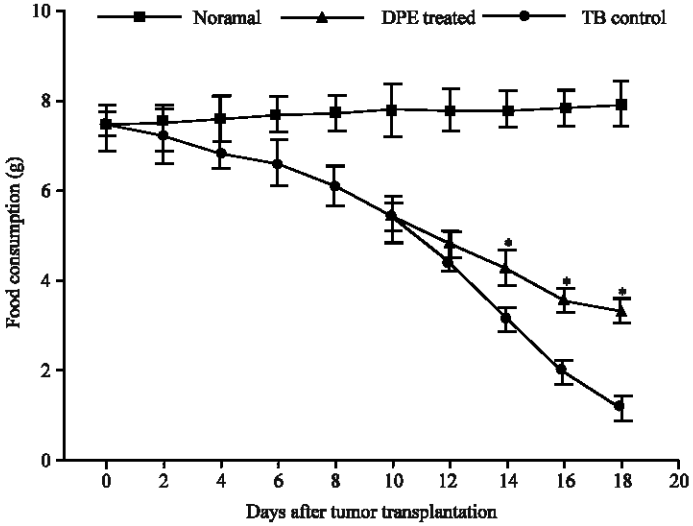

Fig. 3: Graph showing changes in the food consumption of normal, tumor-bearing control and DPE treated mice (20 mg kg^{-1} body wt.). The results are expressed as Mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control, *$p \leq 0.05$

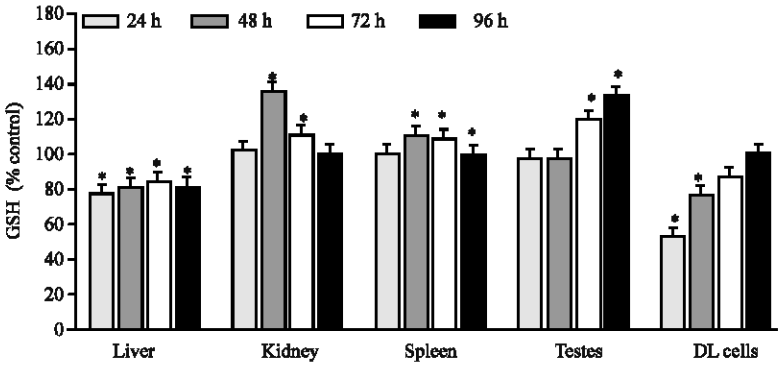

Fig. 4: Histogram showing the percent changes of GSH content (μmoles g^{-1} tissue wet wt.) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg kg^{-1} body wt.). Results are expressed as Mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control (100%), *$p \leq 0.05$

As compared to the respective tissues of normal mice, a gradual decrease in the concentration of GSH was observed in the tissues of tumor-bearing mice (Table 1). DPE treatment caused a significant decrease in GSH concentration in liver and DL cells during 24-96 and 24-48 h of treatment, respectively while a significant increase was observed in kidney, spleen and testes (Table 1). Among the tissues studied, maximum percentage increase in GSH level was noted in kidney (%ILS~137.34) during 48 h of treatment and a maximum decrease was observed in DL cells (%ILS~53.78) during 24 h of treatment (Fig. 4).

As compared to the tissues of normal mice, GST activity of tumor-bearing mice increased in the tissues. DPE treatment of tumor-bearing mice caused a significant increase in GST activity in kidney, spleen, testes and DL cells while a decreased activity was noted in liver (Table 2). Among the tissues

Table 1: Changes in the total GSH content (μmoles g^{-1} tissue wet wt.) in the tissues of normal and tumor-bearing mice under different treatment conditions

Treatments	Liver	Kidney	Spleen	Testes	DL cells
Normal	13.52±0.37	8.19±0.95	9.75±0.44	10.52±0.28	-
TB					
Day 5	12.82±0.25	8.06±0.36	9.46±0.46	9.55±0.41	3.56±0.35
Day 10	11.63±0.51	7.90±0.62	8.86±0.45	9.43±0.27	5.96±0.28
TB control					
24 h	11.51±0.25	7.86±0.26	8.75±0.16	9.37±0.09	5.82±0.35
48 h	10.62±0.41	7.47±0.58	7.73±0.19	8.16±0.10	5.73±0.24
72 h	09.31±0.48	7.32±0.50	7.21±0.11	7.08±0.11	5.31±0.12
96 h	08.56±0.39	6.96±0.24	7.04±0.14	6.65±0.22	4.68±0.05
DPE treated					
24 h	9.01±0.51*	8.12±0.40	8.82±0.18	9.30±0.11	3.13±0.20*
48 h	8.61±0.27*	10.26±0.38*	8.59±0.15*	8.09±0.11	4.52±0.17*
72 h	7.89±0.41*	8.22±0.30*	7.96±0.16*	8.61±0.13*	4.67±0.52
96 h	7.01±0.25*	7.04±0.28	7.11±0.12*	9.03±0.22*	4.77±0.31

Normal = Tissues from hosts without tumor or DPE treatment; TB = Tissues from tumor-bearing mice; TB control = Tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg kg^{-1} body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are expressed as mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control, *$p \leq 0.05$

Table 2: Glutathione s-transferase activity (μmoles min^{-1} mg^{-1} protein) in the tissues of normal and tumor-bearing mice under different treatment conditions

Treatments	Liver	Kidney	Spleen	Testes	DL cells
Normal	0.46±0.02	0.26±0.02	0.17±0.01	0.31±0.02	-
TB					
Day 5	0.55±0.03	0.26±0.01	0.18±0.02	0.35±0.01	0.20±0.01
Day 10	0.68±0.04	0.27±0.02	0.18±0.01	0.37±0.01	0.22±0.01
TB control					
24 h	0.69±0.02	0.26±0.01	0.18±0.01	0.37±0.01	0.23±0.01
48 h	0.62±0.01	0.27±0.01	0.19±0.01	0.39±0.02	0.26±0.01
72 h	0.58±0.02	0.27±0.01	0.20±0.01	0.45±0.02	0.28±0.01
96 h	0.54±0.02	0.29±0.01	0.21±0.01	0.48±0.02	0.31±0.01
DPE treated					
24 h	0.52±0.02*	0.32±0.02*	0.22±0.01*	0.46±0.02*	0.24±0.01*
48 h	0.35±0.02*	0.33±0.01*	0.22±0.01*	0.46±0.02*	0.28±0.01*
72 h	0.41±0.02*	0.28±0.01	0.21±0.01	0.45±0.02	0.32±0.01*
96 h	0.54±0.02	0.28±0.01	0.20±0.02	0.47±0.02	0.33±0.01*

Normal = Tissues from hosts without tumor or DPE treatment; TB = Tissues from tumor-bearing mice; TB control = Tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg kg^{-1} body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are expressed as mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control, *$p \leq 0.05$

studied, maximum percentage increase in GST activity was noted in testes (%ILS~125.75) during 24 h of treatment while maximum percentage decrease was noted in liver (%ILS~56.27) during 48 h of treatment (Fig. 5). During the early stage of tumor growth GR activity increases in the tissues studied which was followed by a gradual decrease during 11 to 14 days of tumor growth except in testes (Table 3). DPE treatment significantly decreased GR activity in liver, spleen and DL cells while a significant increase was observed in kidney. Testes did not show significant changes in GR activity after DPE treatment (Table 3). Maximum percentage increase in GR activity was observed in kidney (%ILS~145.12) during 96 h of treatment and maximum decrease was also observed in spleen (%ILS~42.89) during 24 h of treatment (Fig. 6). As compared to the normal mice, GPx activity of tumor-bearing mice decreased in the tissues studied and DPE treatment resulted in further significant decrease in all the tissues (Table 4). The most predominant percentage decrease in GPx activity was noted in kidney (%ILS~27.82) during 24 h of treatment (Fig. 7).

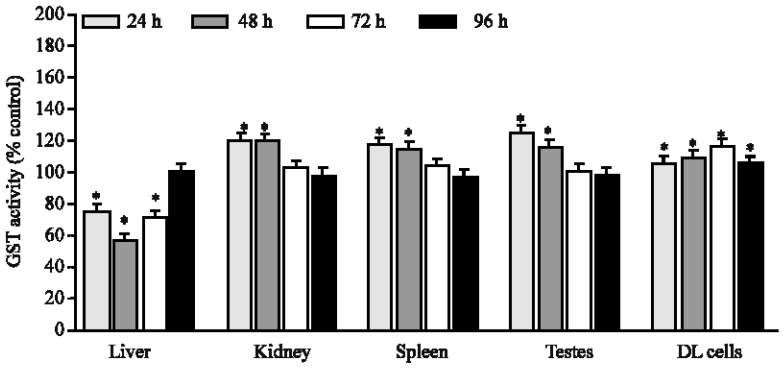

Fig. 5: Histogram showing the percent changes in the specific activity of GST (μmoles min^{-1} mg^{-1} protein) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg kg^{-1} body wt.). Results are expressed as mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control (100%), *p≤0.05

Table 3: Glutathione reductase activity (μmoles min^{-1} mg^{-1} protein) in the tissues of normal and tumor-bearing mice under different treatment conditions

Treatments	Liver	Kidney	Spleen	Testes	DL cells
Normal	0.33±0.02	0.48±0.04	0.21±0.03	0.25±0.02	-
TB					
Day 5	0.36±0.03	0.60±0.04	0.27±0.03	0.26±0.03	0.21±0.02
Day 10	0.40±0.03	0.66±0.06	0.38±0.05	0.27±0.01	0.23±0.02
TB control					
24h	0.43±0.03	0.73±0.06	0.41±0.04	0.29±0.02	0.21±0.01
48h	0.41±0.03	0.71±0.05	0.32±0.02	0.34±0.02	0.18±0.01
72h	0.38±0.03	0.67±0.08	0.23±0.02	0.39±0.04	0.16±0.01
96h	0.36±0.03	0.62±0.06	0.21±0.03	0.47±0.03	0.15±0.01
DPE treated					
24h	0.30±0.02*	0.78±0.06	0.17±0.02*	0.26±0.03	0.14±0.02*
48h	0.26±0.02*	0.84±0.05*	0.21±0.02*	0.31±0.03	0.15±0.01*
72h	0.22±0.02*	0.88±0.04*	0.25±0.02	0.36±0.02	0.16±0.01
96h	0.20±0.03*	0.90±0.07*	0.20±0.03	0.44±0.03	0.15±0.01

Normal = Tissues from hosts without tumor or DPE treatment; TB = Tissues from tumor-bearing mice; TB control = Tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg kg^{-1} body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are expressed as mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control, *p≤0.05

Table 4: Glutathione peroxidase activity (μmoles min^{-1} mg^{-1} protein) in the tissues of normal and tumor-bearing mice under different treatment conditions

Treatments	Liver	Kidney	Spleen	Testes	DL cells
Normal	0.33±0.01	0.13±0.01	0.22±0.01	0.22±0.01	-
TB					
Day 5	0.29±0.02	0.12±0.01	0.20±0.02	0.20±0.02	0.21±0.02
Day 10	0.25±0.02	0.11±0.01	0.19±0.01	0.19±0.01	0.20±0.02
TB control					
24 h	0.22±0.01	0.11±0.01	0.18±0.01	0.19±0.01	0.20±0.07
48 h	0.18±0.01	0.11±0.01	0.16±0.01	0.17±0.02	0.18±0.01
72 h	0.14±0.01	0.09±0.01	0.15±0.01	0.16±0.02	0.17±0.01
96 h	0.09±0.01	0.08±0.01	0.14±0.01	0.15±0.01	0.16±0.01
DPE treated					
24 h	0.14±0.01*	0.03±0.01*	0.14±0.02*	0.08±0.01*	0.11±0.01*
48 h	0.11±0.01*	0.06±0.01*	0.09±0.02*	0.08±0.02*	0.13±0.02*
72 h	0.09±0.01*	0.07±0.02*	0.08±0.01*	0.06±0.01*	0.14±0.02*
96 h	0.07±0.01*	0.08±0.01	0.05±0.01*	0.07±0.01*	0.16±0.01

Normal = Tissues from hosts without tumor or DPE treatment; TB = Tissues from tumor-bearing mice; TB control = Tissues from untreated tumor-bearing hosts receiving extract vehicle alone; DPE = *D. pentagyna* extract, was administered i.p. (20 mg kg^{-1} body wt.) to tumor-bearing mice; DL = Dalton's lymphoma. The results are expressed as mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control, *p≤0.05

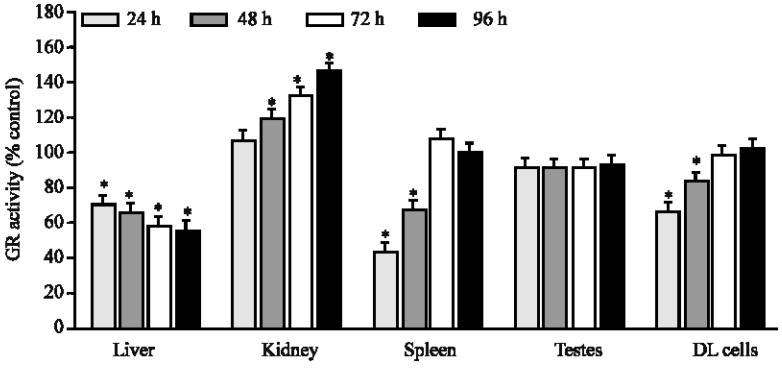

Fig. 6: Histogram showing the percent changes in the specific activity of GR (μmoles min^{-1} mg^{-1} protein) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg kg^{-1} body wt.). Results are expressed as Mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control (100%), *p≤ 0.05

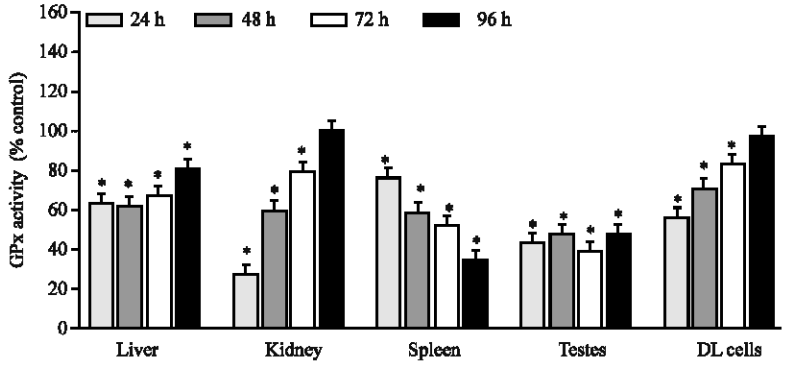

Fig. 7: Histogram showing the percent changes in the specific activity of GPx (μmoles min^{-1} mg^{-1} protein) in the tissues and DL cells of tumor-bearing mice after DPE treatment (20 mg kg^{-1} body wt.). Results are expressed as Mean±SD. Student's t-test, n = 6, as compared to the corresponding TB control (100%), *p≤0.05

DISCUSSION

In the antitumor studies, ascites Dalton's lymphoma has been commonly used as an important murine experimental tumor model (Prasad and Giri, 1994; Nicol and Prasad, 2002). The host survival data indicate significant increase in survivability of tumor-bearing mice (% ILS≤20) treated with DPE at a dose of 10, 15, 20, 25 and 30 mg kg^{-1} body wt. day^{-1} as compared to the control tumor-bearing mice suggesting its antitumor potentials with the most potent antitumor activity (%ILS~70) at the dose of 20 mg kg^{-1} body wt. day^{-1}. As compared to the regular increase in the body weight of tumor-bearing control mice, the observed significant decrease in the body weight of DPE-treated mice may indicate an inhibitory effect of *D. pentagyna* on tumor growth. In DPE-treated tumor-bearing mice, the food consumption was noted to be much better than that in the tumor-bearing control mice.

Present studies showed variations in GSH concentrations in different tissues of normal and tumor-bearing mice. As compared to the tissues of normal mice, GSH concentrations decreased in the tissues of tumor-bearing mice studied except DL cells. The increase of GSH in DL cells with tumor growth

i.e., from day 5 to day 10 of tumor transplantation, could be involved in facilitating the proliferation and metabolism of tumor cells in the hosts as it has been reported that GSH controls the onset of tumor cell proliferation by regulating protein kinase C activity and intracellular pH (Terradez *et al*., 1993).

The observed increase of GSH level in DL cells became an investigating interest to find the changes in the GSH level of various tissues, if any, after *Dillenia pentagyna* extract (DPE) treatment. DPE treatment of tumor-bearing hosts showed differential pattern of effects in different tissues. The observed DPE-mediated increase in GSH level in kidney, spleen and testes of tumor-bearing host may have a role in protection of these tissues against oxidative damage caused by free radicals. However, the decrease in GSH level, particularly in DL cells by DPE treatment, may be a noteworthy step in the antitumor activity of DPE against Dalton's lymphoma. The harmful effect of free radicals in various tissues of the body are controlled by antioxidant defense system of the cells, of which the most important free radical chain breaking molecule is glutathione (Erat *et al*., 2007). Depletion of cellular GSH by buthionine sulfoximine (BSO) has been shown to sensitize tumor cells to oxidative stress, irradiation and certain chemotherapeutic agents *in vitro* (Navarro *et al*., 1999; Mitchell *et al*., 1983; Schnelldorfer *et al*., 2000). Therefore, it can be suggested that, DPE-mediated decrease in GSH in DL cells may have a role in the antitumor activity of *Dillenia pentagyna* by decreasing cellular antioxidant-mediated protective mechanism, thus increasing DL cell's susceptibility to cell death. Among the normal tissue studied, decrease level of GSH was observed mainly in liver. Since liver is the major site of glutathione metabolism and glutathione content is highest in the liver, some decrease of glutathione level in liver after DPE treatment may still be sufficient to protect the tissue from damage etc. However, studies on the evaluation of various possible toxic effect of *D. pentagyna* in the hosts could be the area of some research interest.

GSTs are major phase II detoxification enzymes found mainly in the cytosol. In addition to their role in catalyzing the conjugation of electrophilic substrates to glutathione (GSH), these enzymes also carry out a range of other functions. They have peroxidase and isomerase activities and are involved in protection of cells against H_2O_2-induced cell death (Sheehan *et al*., 2001). Therefore, from the present finding of DPE-mediated increase in GST activity in some tissues (particularly kidney, spleen and testes), it may be suggested that this increase in GST activity may also have a role in the cellular defence. GR plays an essential role in the cellular defense against oxidative stress and a controlled decrease of GR level in human fibroblasts was reported to reduce cell viability (Chavkova *et al*., 2001). When GR activity is impaired, the ability of the cell to reduce GSSG to GSH may be hampered, leading to GSSG accumulation within the cytosol. GPx/GR system is also critical as a cellular defence mechanism against oxidizing species such as hydrogenperoxide (H_2O_2) and hydroperoxides of fatty acids and phospholipids formed as a result of reactive oxygen species (Li *et al*., 2000; Ekholm and Bjorkman, 1997; Wakimoto *et al*., 1998). While a decrease level of GR and GPx in some normal tissues may cause to weaken the cellular defense mechanism, the observed DPE-mediated decrease in GR activity in DL cells could be one of the possible steps involved to decrease GSH level in DL cells, thus, affecting DL cell's antioxidant machinery and reduced cell viability. It may also be suggested that decreased GPx activity in DL cells may allow accumulation of free radicals inside the cell leading to tumor cell death, thereby increasing host survivability.

Out of different dose of DPE used in the present study, 20 mg kg^{-1} body wt. day^{-1} exhibited the most effective antitumor potential against murine ascites Dalton's lymphoma. DPE-mediated decrease in the level of GSH and activities of GR and GPx in DL cells may cause to diminish the protective ability of tumor cells, thereby facilitating tumor cell death. Thus, it may be suggested that, changes in GSH and GSH-related enzymes in tumor cells should be an important contributory factor in *Dillenia pentagyna*-mediated antitumor activity.

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