



## ANTICANCER AND ANTIOXIDANT PROPERTIES OF ALKALOID EXTRACT OF *SOLANUM KHASIANUM* BERRIES.

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### ABSTRACT

In the present studies the anticancer properties of alkaloidal extract from *Solanum khasianum* berries was investigated against murine ascites Dalton's lymphoma *in vivo*. *In vitro* radical scavenging activity of the alkaloid extract against 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical was also determined. The results of present study showed that

alkaloidal extract (SKF-Alk) exhibited potent anticancer activity against Dalton's lymphoma *in vivo* in a dose-dependent manner. Maximum anticancer activity (%ILS = 185%) was observed at a dose of 60 mg/kg/day. A single intraperitoneal injection of tumor-bearing mice with SKF-Alk (60 mg/kg b. wt.) caused significant decrease in tissue glutathione content and significant increase in tissue lipid peroxidation and catalase activities. SKF-Alk-mediated decrease in GSH level, catalase activity and increase in lipid peroxidation in DL cells and the antioxidant property of this alkaloidal extract may be an important contributory factors involved in its anticancer potential. However, further studies are required to establish its anticancer efficacy using more animal and human cancer cell lines and also to observe any adverse side effects in the normal tissues.

**KEYWORDS:** *Solanum khasianum*, alkaloid, Dalton's lymphoma, anticancer, radical scavenging.

### INTRODUCTION

It has been realized that many human diseases are the result of oxidative stress that results from an imbalance between formation and neutralization of pro-oxidants. Oxidative stress is initiated by free radicals such as hydroxyl, peroxy and superoxide radicals, which become stable through electron pairing with biological macromolecules such as proteins, lipids and

DNA in healthy human cells and cause protein and DNA damage along with lipid peroxidation. The damage caused by oxidative stress has been implicated as a potential contributor to the pathogenesis of cancers and many more diseases.<sup>[1,2]</sup> The tissue or cellular damage can become more widespread due to weak antioxidant defense systems. All biological systems have antioxidant defense mechanism that protects against oxidative damages and repairs enzymes to remove damaged molecules. In some cases, this natural antioxidant defense mechanism can be inefficient; hence dietary intake of antioxidant is required. Antioxidants are substances that prevent cells from free radical attacks. It also turns free radicals into waste by products, and they eventually get eliminated from the body. However, consumption of fruits and vegetables rich in fibers and vitamins is known to help protect cells from the risk of several diseases, such as cancer, cardiovascular diseases and stroke caused by oxidative stress.<sup>[3,4]</sup>

Reduced glutathione, an endogenous intracellular thiol-containing tripeptide (L- $\gamma$ -glutamyl-L-cysteinyl-glycine, GSH), is an important cellular antioxidant. It is ubiquitous in animals, plants and microorganisms.<sup>[5]</sup> Under normal physiological conditions, mammalian cells maintain more than 98% of intracellular GSH in the reduced form (GSH) with the help of GSSG reductase and maintain an intracellular concentration of 0.5 to 10 mM. The key functional element of GSH is the cysteinyl moiety, which provides the reactive thiol group and is responsible for the many functions of GSH 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.<sup>[6]</sup> Therefore, GSH has been the focus of interest in cancer chemotherapy.<sup>[7]</sup> Lipid peroxidation, the oxidative breakdown of polyunsaturated fatty acids, is widely accepted as a general mechanism for cellular injury and death, and is a major contributor to the loss of cell function under oxidative stress situations. Lipid peroxidation can alter vital membrane protein structure and function. The presence of lipid hydro peroxides in a membrane disrupts its function by altering fluidity and allowing ions such as  $\text{Ca}^{++}$  to leak across the membrane, the consequences of which include activation of phospholysis, membrane blebbing and eventual membrane rupture. The antioxidants in such cases can act as stabilizers of homeostasis.

It became an investigating interest of the research to explore the naturally occurring effective anticancer agents that would prevent, slow, or reverse cancer development. The anticancer

efficacy of aqueous extract of *Solanum khasianum* fruit have been reported earlier and the phytochemical analysis also revealed the presence of alkaloids in the extract.<sup>[8]</sup> Alkaloids have a wide distribution in the plant kingdom and mainly exist in higher plants. They are important chemical compounds that serve as a rich reservoir for drug discovery. Several alkaloids isolated from natural herbs exhibit antiproliferation and antimetastasis effects on various types of cancers both *in vitro* and *in vivo*. Alkaloids, such as camptothecin and vinblastine, have already been successfully developed into anticancer drugs.<sup>[9,10]</sup> Several studies also indicate that combination therapy probably provides an optimal venue for the clinical application of compounds because most of the alkaloids exhibit synergistic or enhancement effects when combined with chemotherapeutic drugs in both *in vitro* and *in vivo* experiments.<sup>[11, 12, 13, 14]</sup> Therefore, taking into consideration the above facts, an attempt has been made to evaluate the anticancer and antioxidant properties of alkaloidal extract of *Solanum khasianum* berries.

## MATERIALS AND METHODS

### Alkaloid extraction

Extraction of alkaloids from the berries of *Solanum khasianum* was done following the methods described by Bhattacharya *et al.* (2013)<sup>[15]</sup> and Almazini *et al.* (2009)<sup>[16]</sup> with some modifications. Briefly, 200 g of dried powder of *Solanum khasianum* berries was transferred into the Soxhlet apparatus and then defatted with petroleum ether to yield greenish yellow oil, which is rejected as it is devoid of glycoalkaloid. Defatted material was then transferred into a round-bottom flask and gently shaken for overnight in 3 L of 2% aqueous oxalic acid in a magnetic stirrer apparatus at room temperature. The mixture was filtered through Whatman No. 1 filter paper and the clear filtrate (1700ml) was collected. The filtrate was then gently heated at 75°C and alkalized by adding 85ml of 60% NaOH. The mixture was kept cool at room temperature and then transferred into the refrigerator (15°C) and left overnight. Precipitate formed was collected by centrifugation at 20°C for 10 minutes at 3000 rpm. The collected precipitate was refluxed in 450 ml of 0.5 M HCl for 90 minutes at 100°C. The mixture was cooled and again alkalized by adding 85 ml of 60% NaOH and then heated at 100°C for 15 minutes. After cooling, the mixture was centrifuged as above and the brownish pellet was collected and dried at 70°C. The dried pellet was then dissolved in hot absolute methanol and the colored impurities were removed by using activated charcoal. The resultant yellowish white extract (SKF-Akl) was evaporated to dryness and tested for the presence of alkaloids and its cytotoxic activity *in vitro*.

### Animals & tumor maintenance

Inbred Swiss albino mice colony is being maintained under laboratory conditions keeping 5-6 animals in a propylene cage at 23-25°C. The animals were fed with commercially available food pellets and water *ad libitum*. Ascites Dalton's lymphoma (DL) tumor was maintained *in vivo* in 10–12 weeks old mice by serial intraperitoneal (i.p.) transplantation of  $1 \times 10^7$  viable tumor cells per animal (in 0.25 ml phosphate buffered saline, pH 7.4). Tumor-transplanted mice usually survive for 20 days.

### Anticancer studies

*In vivo* anticancer activity of SKF-Alk was studied following the method described by Sakagami et al. (1987)<sup>[17]</sup>  $1 \times 10^7$  viable DL 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. 0.25 ml of SKF-Alk (dissolved in PBS) was given intraperitoneally for 7 consecutive days starting from day 1 of tumor transplantation and the host survival patterns were recorded. Different doses of SKF-Alk (5, 10, 20, 30, 40, 50, 60 and 75 mg/kg/day) was used for the study and the anticancer efficacy was reported in percentage of average increase in life span (%ILS) calculated using the following formula

$$\% \text{ILS} = (T/C \times 100) - 100$$

where, T and C are the mean survival days of treated and control animals respectively. Control and different groups of mice with different doses consisted of 10 mice each.

### Treatment groups

Based on the result of *in vivo* anticancer studies, 50 mg/kg b. wt. dose of the SKF-Alk was selected for use in the biochemical and enzymatic studies. Animals were divided into three groups. **Group I** consisted of normal mice without tumor. **Group II** consisted of tumor-bearing control receiving 0.25 ml of extract vehicle alone (PBS) and **Group III** consisted of tumor-bearing mice receiving a single intraperitoneal (i.p.) injection of SKF-Alk on the 10<sup>th</sup> day of post-tumor transplantation which is the logarithmic phase of tumor growth.

Group II and III animals were sacrificed by cervical dislocation on the 11, 12 and 13<sup>th</sup> day of tumor growth, i.e., 24, 48 and 72 hours of treatment. Liver, kidney, testes and DL cells were collected and used for determination of protein concentration, glutathione, lipid peroxidation and catalase activities.

### **Protein estimation**

The protein content of different tissues was determined following the method of Lowry et al. (1951)<sup>[18]</sup> using bovine serum albumin (BSA) as standard. Tissue homogenate (2%) was prepared in 0.25M sucrose solution. To 1 ml of the homogenate taken in duplicate, 5 ml of freshly prepared protein reagent [49 ml of 2% Na<sub>2</sub>CO<sub>3</sub> in 0.1M NaOH + 1 ml of Copper sulphate, Sodium- Potassium tartarate solution (1% CuSo<sub>4</sub>.5H<sub>2</sub>O in 1% Na-K tartarate)] was added, thoroughly mixed and allowed to stand at room temperature exactly for 10 minutes. After 10 minutes, 0.5 ml of 1N Folin-Ciocalteau reagent was added and mixed instantly. The reaction mixture was then kept at room temperature for 30 minutes to complete the reaction and the optical density was read at 750 nm against reagent blank. The protein concentration was determined from the standard curve obtained by using bovine serum albumin (BSA) as standard.

### **Glutathione determination**

Glutathione level was determined as total sulfhydryl contents using the method of Sedlak and Lindsay (1968).<sup>[19]</sup> Briefly, 5% tissue homogenates were prepared in 0.02M EDTA (pH 4.7) in a motor-driven teflon–pestle homogenizer. Total glutathione was determined by adding the homogenate (0.1 ml) to 1 ml of 0.2 M Tris–EDTA buffer (pH 8.2) and 0.9 ml of 0.02 M Tris–EDTA buffer (pH 4.7) followed by 20 µl of Ellman’s reagent. After 30 minutes of incubation at room temperature, the reaction mixture was centrifuged at 3000xg for 20 minutes and the absorbance of the clear supernatant was read against a reagent blank at 412 nm in a spectrophotometer.

### **Lipid peroxidation assay**

Lipid peroxidation in different tissue samples was determined using the method described by Buege and Aust (1978).<sup>[20]</sup> Briefly, 10% tissue homogenate was prepared in 0.15M KCl. To 1 ml of the tissue homogenate, 2 ml of the trichloro acetic acid (15%)-thiobarbituric acid (0.375%)-HCl (0.25N) reagent was added and mixed thoroughly. The sample was then heated in a boiling water bath for 15 minutes, and then cooled at room temperature. The precipitate was removed by centrifugation at 1000xg for 10 minutes. The absorbance of the clear supernatant was then read at 535nm against the blank. The malondialdehyde (MDA) concentration of the tissue sample was calculated using an extinction coefficient of  $1.56 \times 10^5 \text{ M}^{-1} \text{ cm}^{-1}$  and expressed as nmol of MDA/mg protein.

### Catalase activity assay

Catalase activity was assayed according to Aebi (1984).<sup>[21]</sup> Briefly, the assay volume (3.0 ml) contained 20 µl tissue homogenate (5%), 1.98 ml of 50 mmol/L phosphate buffer (pH 7.0). Adding 1.0 ml of 30 mmol/L H<sub>2</sub>O<sub>2</sub> started the reaction maintained at 20°C. The decrease in absorbance at 240 nm was monitored for 60 seconds in a spectrophotometer. The enzyme activity was calculated using the extinction coefficient of 0.00394 L mmol<sup>-1</sup> mm<sup>-1</sup> and the unit of enzyme specific activity was expressed as µmoles of H<sub>2</sub>O<sub>2</sub> decomposed per minute per mg protein.

### *In vitro* radical scavenging activity

The radical scavenging activity of SKF-Alk against 2,2-Diphenyl-1-picryl hydrazyl (DPPH) radical was determined using the method described by Ayoola et al. (2008).<sup>[22]</sup> Briefly, different concentrations of the extract were prepared. Ascorbic acid was used as the antioxidant standard at concentrations of 50, 100, 500 and 1000 µg/ml. 1 ml of the extract was placed in a test tube, and 3 ml of methanol was added followed by 0.5 ml of 1 mM DPPH in methanol. The radical scavenging activity was calculated using the following formula

$$\% \text{ scavenging} = \{[A_b - A_a]/A_b\} \times 100$$

where, A<sub>b</sub> and A<sub>a</sub> are the absorbance of the blank sample and test/extract sample respectively.

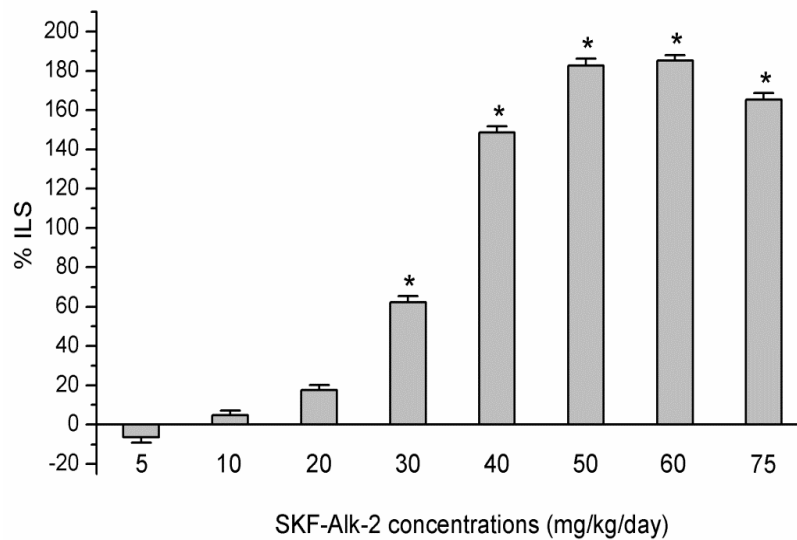
### Statistical analysis

All statistical analysis was done using statistical software 'OriginPro 8 SRO v8.0724 (B724), Northampton, MA, USA'.

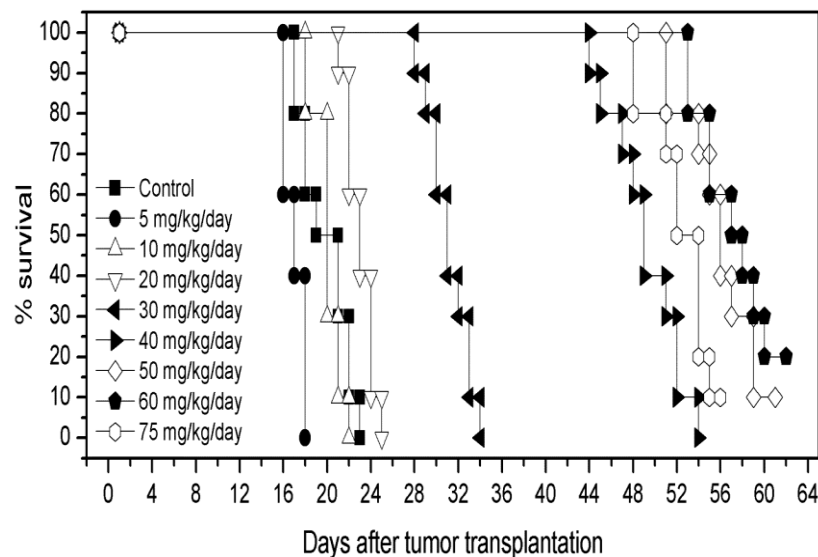
## RESULTS

In the *in vivo* anticancer studies, ascites Dalton's lymphoma has been commonly used as an important murine experimental cancer/tumor model.<sup>[23]</sup> The effect of SKF-Alk on the survivability of the hosts in different experimental groups has been described in Figure 1. The death of mice was recorded daily and the survival pattern of mice in different experimental groups was determined. Out of eight doses used in the present study, 60 mg/kg/day showed comparatively better antitumor activity against ascites Dalton's lymphoma. It showed 185 %ILS at a dose of 60 mg/kg/day. Significant increase in the %ILS was observed with the higher five doses used, i.e., 30 to 75 mg/kg/day. The survival patterns of tumour-bearing mice treated with different doses of SKF-Alk has also been compared (Fig. 2). The result

showed that maximum survival of the hosts was observed with 60 mg/kg/day where 100% animals survived for 53 days and 20% animals survived for more than 60 days.



**Figure 1. Histogram showing the anticancer activity of SKF-Alk against murine ascites Dalton's lymphoma *in vivo*. \*%ILS  $\geq$  20%. Results are mean  $\pm$  S.D.**



**Figure 2. Kaplan Mayer's estimate of survival of DL tumor-bearing mice treated with different doses of SKF-Alk. Results are expressed as mean of 6 independent experimental sets.**

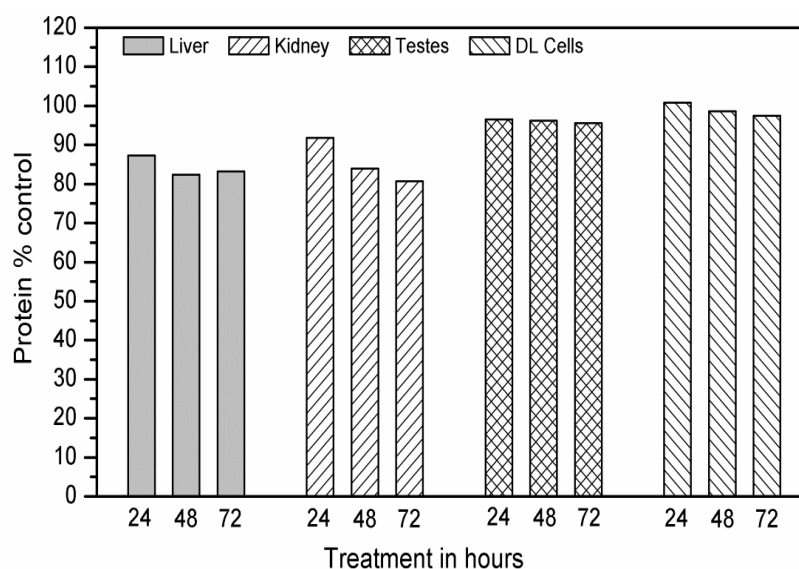
Among different tissues of normal mice studied, the highest protein concentration was observed in liver (303.57 mg/g tissue) followed by kidney (209.27 mg/g tissue) and testes (162.46 mg/g tissue) (Table 1). As compared to normal mice, protein content of liver and testes in tumor-bearing control decreased significantly, while in kidney significant increase

was noted. There was no significant change in the protein content of DL cells during 11 to 13 days of tumor growth. As compared to the corresponding tumor-bearing controls, a significant decrease in the protein content was noted in liver and kidney after SKF-Alk treatment. However, testes and DL cells did not show significant changes in the protein content during 72 h of extract treatment. In all the tissues studied, the maximum decrease in protein content was observed during 72 h of treatment except in liver where maximum decrease was noted during 48 h of treatment (Fig. 3).

**Table 1. Total protein concentrations (mg/g tissue) in the tissues of tumor-bearing mice after SKF-Alk single treatment (60 mg/kg b. wt.).**

Treatment conditions	Liver	Kidney	Testes	DL cells
Normal	303.57 ± 6.1	209.27 ± 7.3	162.46 ± 7.5	-
Control - 24h	270.55 ± 6.3	217.17 ± 4.3	146.77 ± 5.1	151.42 ± 4.2
- 48h	266.48 ± 5.4	223.41 ± 5.1	141.59 ± 5.2	150.33 ± 3.7
- 72h	256.82 ± 4.8	229.72 ± 4.7	137.27 ± 5.7	148.49 ± 3.9
Treated - 24h	236.32 ± 5.2*	199.46 ± 4.9*	141.67 ± 4.2	150.63 ± 4.7
- 48h	219.47 ± 5.4*	187.48 ± 5.1*	136.28 ± 4.5	148.24 ± 4.6
- 72h	213.62 ± 4.8*	185.36 ± 4.6*	131.22 ± 5.1	144.73 ± 4.1

\* Significance between treated and control groups. Results are mean ± S.D. Student's *t*-test, N = 6, *p* < 0.5.



**Figure 3. Histogram showing the percent changes in the protein content in the tissues of tumor-bearing mice after SKF-Alk treatment (60 mg/kg b. wt.).**

Comparison of GSH level among the tissues of normal mice showed the highest glutathione concentration in liver (13.22  $\mu\text{mol/g}$  tissue) followed by testes (9.73  $\mu\text{mol/g}$  tissue) and

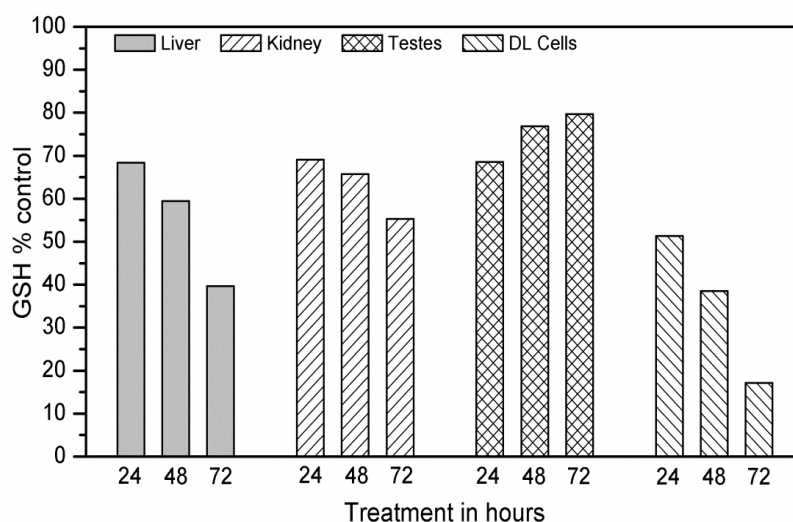


kidney (8.04  $\mu\text{mol/g}$  tissue) (Table 2). As compared to the corresponding tissue of normal mice, the glutathione concentration in liver, kidney and testes of tumor-bearing control significantly decreased. SKF-Alk treatment resulted in a significant decrease in the total GSH concentration in liver, kidney, testes and DL cells during 24 to 72 h of treatment. Maximum decrease in the level of GSH was observed during 72 h of treatment in liver, kidney and DL cells and 24 h of treatment in testes (Fig. 4).

**Table 2. Changes in the total reduced glutathione content ( $\mu\text{mol/g}$  tissue) in the tissues of tumor-bearing mice after single treatment with SKF-Alk (60 mg/kg b. wt.).**

Treatment conditions	Liver	Kidney	Testes	DL cells
Normal	13.22 $\pm$ 0.32	8.04 $\pm$ 0.24	9.73 $\pm$ 0.26	-
Control - 24h	11.28 $\pm$ 0.23	7.32 $\pm$ 0.17	9.15 $\pm$ 0.11	5.34 $\pm$ 0.23
- 48h	10.32 $\pm$ 0.33	7.18 $\pm$ 0.21	8.04 $\pm$ 0.13	5.27 $\pm$ 0.12
- 72h	09.13 $\pm$ 0.35	7.09 $\pm$ 0.28	7.18 $\pm$ 0.16	5.25 $\pm$ 0.14
Treated - 24h	07.71 $\pm$ 0.21*	5.06 $\pm$ 0.31*	6.27 $\pm$ 0.14*	2.74 $\pm$ 0.11*
- 48h	06.14 $\pm$ 0.27*	4.72 $\pm$ 0.25*	6.18 $\pm$ 0.21*	2.03 $\pm$ 0.15*
- 72h	03.62 $\pm$ 0.21*	3.92 $\pm$ 0.22*	5.72 $\pm$ 0.17*	0.90 $\pm$ 0.12*

\* Significance between treated and control groups. Results are mean  $\pm$  S.D. Student's *t*-test, N = 6,  $p < 0.5$ .



**Figure 4. Histogram showing the percent changes in the glutathione concentrations of different tissues of tumor-bearing mice after SKF-Alk treatment (60 mg/kg b. wt.).**

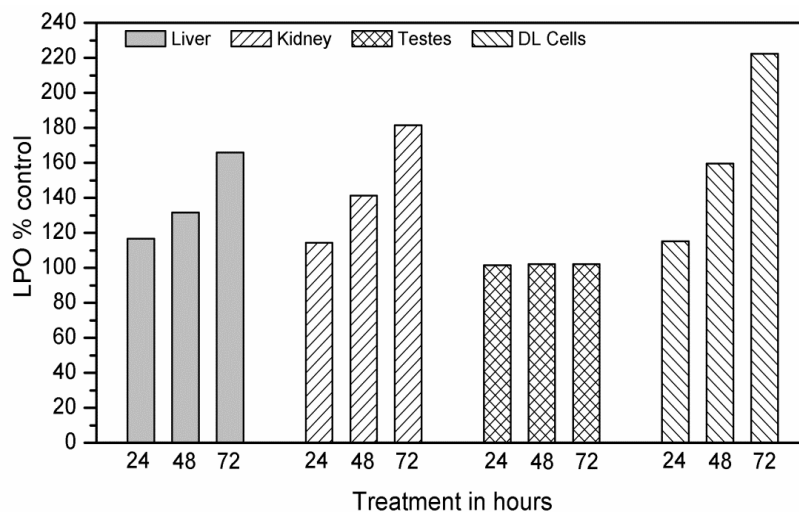
The lipid peroxidation (LPO) was measured in terms of malondialdehyde concentrations in different tissues of mice. In the normal mice, the highest LPO was noted in testes (0.197 nmol/mg protein) followed by kidney (0.172 nmol/mg protein) and liver (0.137 nmol/mg

protein) (Table 3). As compared to the corresponding normal tissues, a significant increase in the level of LPO was observed in all the tissues during tumor growth progression. As compared to their corresponding controls, SKF-Alk treatment significantly increased LPO in liver, kidney and DL cells. The maximum increase in liver, kidney and DL cells were noted during 72 h of treatment (Fig. 5).

**Table 3. Changes in Lipid peroxidation (nmol/mg protein) in the tissues of tumor-bearing mice after single treatment with SKF-Alk (60 mg/kg b. wt.).**

Treatment conditions	Liver	Kidney	Testes	DL Cells
Normal	0.137±0.013	0.172±0.010	0.197±0.015	-
Control - 24h	0.156±0.014	0.189±0.021	0.322±0.013	0.119±0.010
- 48h	0.168±0.011	0.194±0.014	0.331±0.014	0.114±0.012
- 72h	0.173±0.010	0.205±0.022	0.362±0.015	0.116±0.012
Treated - 24h	0.182±0.013*	0.216±0.021*	0.327±0.013	0.137±0.010*
- 48h	0.221±0.011*	0.274±0.016*	0.338±0.012	0.182±0.011*
- 72h	0.287±0.014*	0.372±0.021*	0.370±0.012	0.258±0.013*

\* Significance between treated and control groups. Results are mean ± S.D. Student's *t*-test, N = 6, *p* < 0.5.



**Figure 5. Histogram showing the percent changes in the level of lipid peroxidation in the tissues of tumor-bearing mice after SKF-Alk treatment (60 mg/kg b. wt.).**

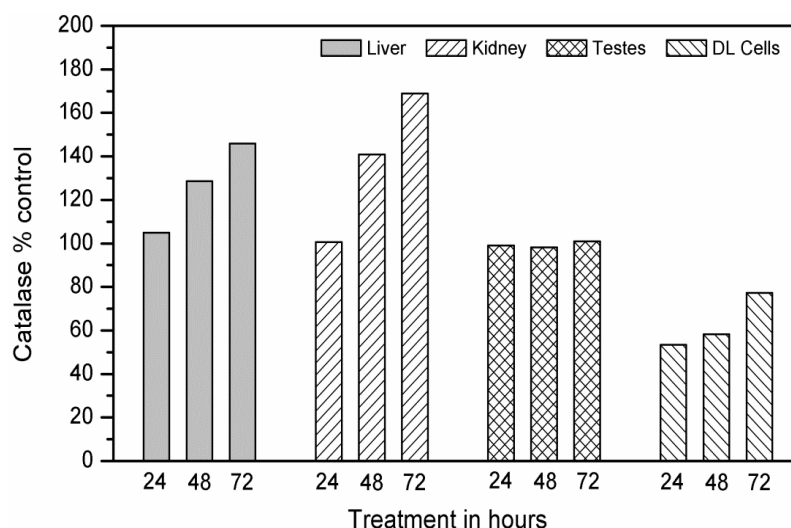
In the normal mice, the highest level of catalase activity was noted in the liver (0.314 nmol/mg protein) followed by kidney (0.152 nmol/mg protein) and testes (0.116 nmol/mg protein) (Table 4). A significant decrease in catalase activity was observed in all the tissues during tumor growth progression. As compared to their corresponding controls, SKF-Alk

treatment caused significant increase in catalase activity of liver and kidney during 48 to 72 h of treatment while a significant decrease was noted in DL cells during 24 to 72 h of treatment. The maximum increase of catalase activity in liver and kidney was noted during 72 h of treatment while maximum decrease in DL cells was noted during 24 h of treatment (Fig. 6).

**Table 4. Changes in catalase activity ( $\mu\text{mol}/\text{min}/\text{mg}$  protein) in the tissues of tumor-bearing mice after single treatment with SKF-Alk (60 mg/kg b. wt.).**

Treatment conditions	Liver	Kidney	Testes	DL Cells
Normal	0.314 $\pm$ 0.023	0.152 $\pm$ 0.020	0.116 $\pm$ 0.015	-
Control - 24h	0.262 $\pm$ 0.021	0.144 $\pm$ 0.016	0.113 $\pm$ 0.017	0.172 $\pm$ 0.021
- 48h	0.227 $\pm$ 0.024	0.105 $\pm$ 0.018	0.114 $\pm$ 0.014	0.177 $\pm$ 0.023
- 72h	0.203 $\pm$ 0.018	0.087 $\pm$ 0.018	0.113 $\pm$ 0.016	0.176 $\pm$ 0.018
Treated - 24h	0.275 $\pm$ 0.017	0.145 $\pm$ 0.021	0.112 $\pm$ 0.017	0.092 $\pm$ 0.017*
- 48h	0.292 $\pm$ 0.022*	0.148 $\pm$ 0.017*	0.112 $\pm$ 0.021	0.103 $\pm$ 0.019*
- 72h	0.296 $\pm$ 0.024*	0.147 $\pm$ 0.019*	0.114 $\pm$ 0.022	0.136 $\pm$ 0.021*

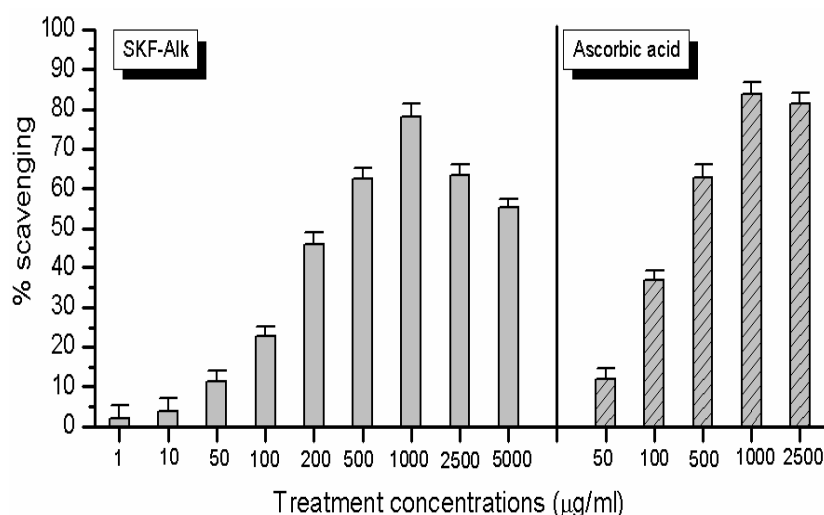
\* Significance between treated and control groups. Results are expressed as Mean  $\pm$  S.D. Student's t- test, n=6, p < 0.05.



**Figure 6. Histogram showing the percent changes in the catalase activity of different tissues of tumor-bearing mice after SKF-Alk treatment (60 mg/kg b. wt.).**

The free radical scavenging activity of SKF-Alk and ascorbic acid was assessed by DPPH assay. SKF-Alk demonstrated ability to inhibit DPPH radical. Among different concentrations tested maximum radical scavenging activity of SKF-Alk (78.16%) was observed at a concentration of 1000  $\mu\text{g}/\text{ml}$ . Radical scavenging activity of positive control,

ascorbic acid (83.74%) was also observed at a concentration of 1000  $\mu\text{g/ml}$ . The results indicated that SKF-Alk and ascorbic acid possesses a more or less similar inhibitory activity.



**Figure 7.** Graph showing the % scavenging activity of SKF-Alk and ascorbic acid *in vitro*. Results are mean  $\pm$  S.D.  $p < 0.05$ .

## DISCUSSION

Ascites Dalton's lymphoma has been commonly used as an important murine experimental tumor model to investigate the anticancer activity.<sup>[23]</sup> The same tumor model was used in the anticancer activity study of SKF-Alk. The host survival data indicate a significant increase in survivability of tumor-bearing mice (%ILS $\geq$ 20%) treated with SKF-Alk at a dose of 30, 40, 50, 60 and 75 mg/kg/day as compared to the control tumor-bearing mice suggesting its anticancer potentials with the most potent anticancer activity (%ILS $\sim$ 185) at a dose of 60 mg/kg/day.

SKF-Alk treatment of DL tumor-bearing hosts caused significant decrease in the protein contents in liver and kidney. These changes may involve some alterations in the rate of protein synthesis or decreased uptake of protein in these tissues. On the other hand, it may be due to some toxic effect in the liver and kidney of the host.

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 all the tissues of tumor-bearing mice (Table 2). GSH and its dependent enzymes work with other antioxidants and antioxidant enzymes to protect cells against reactive oxygen intermediates (ROIs).<sup>[24]</sup> 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. Elevation of intracellular GSH levels has also been suggested to be involved in the resistance of cancer cells to oxidative stress, radiotherapy and chemotherapy,<sup>[25]</sup> while a depletion of GSH levels could increase the cytotoxicity of a variety of antitumor agents which in turn could induce the apoptotic cell death also.<sup>[26, 27]</sup> The observed SKF-Alk -mediated decrease in GSH level in liver, kidney and testes of tumor-bearing host (Table 2) may play a role in the toxic effect of plant extract (SKF-Alk) in these tissues. However, the decrease in GSH level, particularly in DL cells by the SKF-Alk treatment, may be a noteworthy step in the anticancer activity of the alkaloidal extract of *Solanum khasianum* berries against Dalton's lymphoma. Therefore, it can be suggested that, SKF-Alk-mediated decrease in GSH in DL cells may have a role in the anticancer activity of *Solanum khasianum* berries by increasing DL cell's susceptibility to oxidative stress, thereby, increasing host survivability. Studies in human and rodent tumor cell lines have shown that resistance to alkylating agents may, in certain instances, be due to elevated tumor GSH concentrations. Depletion of cellular GSH by buthionine sulfoximine (BSO) has been shown to sensitize tumor cells to irradiation and to certain chemotherapeutic agent *in vitro*.<sup>[28, 29]</sup> Therefore, treatment strategies involving GSH depletion should also be taken into consideration in order to maximize the therapeutic efficacy of anticancer agents and the proposal of combination therapy with *Solanum khasianum* berries extract and other anticancer agents may also be useful in providing better anticancer activity.

Cancer cells can generate large amounts of hydrogen peroxides which may contribute to their ability to damage normal tissues.<sup>[30]</sup> Some chemical drug treatment is associated with induction of oxidative stress by generation of free radicals and reactive oxygen species,<sup>[31, 32]</sup> and the potential role of dietary antioxidants, such as ascorbic acid, tocopherol,  $\beta$ -carotene etc. to reduce the activity of free radical-induced reactions has drawn increasing attention.<sup>[33]</sup> In the present studies, as compared to the corresponding normal tissues, an increased level of lipid peroxidation was observed in liver, kidney and testes of mice during tumor progression (Table 3), and treatment of tumor-bearing mice with SKF-Alk resulted in a significant further increase in the tissues except testes. Therefore, these results may suggest that the SKF-Alk-mediated increase of lipid peroxidation noted in DL cells may play a role in maximizing DL cell damage by oxidative processes.

In an attempt to further understand on the significance of GSH in the anticancer activity of SKF-Alk, the activity of catalase was also assayed in the tissues. Drug treatment induced GSH depletion may account for the enhanced lipid peroxidation.<sup>[34]</sup> Catalase catalyzes decomposition of H<sub>2</sub>O<sub>2</sub> to H<sub>2</sub>O and O<sub>2</sub> and thus protects cells from oxidative damage.<sup>[35]</sup> The results from catalase assay showed that the enzyme activity was significantly reduced in DL cells after SKF-Alk treatment (Table 4). This SKF-Alk-mediated inhibition of catalase activity in the tumor cells should also be helping to increase the lipid peroxidation, which is partially achieved by decrease in GSH.

Reactive oxygen species have multiple functions and are implicated in tumor initiation and progression.<sup>[36]</sup> Depleted endogenous antioxidant enzymes with enhanced free radical generation are well documented in carcinogenesis.<sup>[30]</sup> Many tumor cells have pro-oxidant status and promote oxidative stress. This increases the surviving potentials of the cancer cells by inducing mutations, activating redox signaling and stimulating pro-survival factors such as NF- $\kappa$ B and AP-1.<sup>[37]</sup> Antioxidants alter the intracellular redox state, thereby enhancing the effects of cytotoxic therapy. The significant DPPH scavenging potentials of SKF-Alk observed in the present study proves its antioxidant activity. It was reported that plant-derived extracts containing antioxidant principles showed cytotoxicity toward tumor cells and antitumor activity in experimental animals.<sup>[38]</sup>

In conclusion, results of present study showed a significant anticancer efficacy of alkaloidal extract of *S. khasianum* berries (SKF-Alk) against murine ascites Dalton's lymphoma in a dose-dependent manner. SKF-Alk-mediated decrease in GSH level, catalase activity and increase in lipid peroxidation in DL cells and the antioxidant property of the extract may be an important contributory factors involved in its anticancer potential. However, further studies are required to establish its anticancer efficacy using more animal and human cancer cell lines and also to observe any adverse effects in the normal host tissues.

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