Anti-Tumor Activity of Viper Snake Venom Photo-products SV_1 and SV_2 against Ehrlich Ascites Carcinoma in Mice

Shivaji P. Gawade*, Munaf A. Tamboli and Sachin S. Thorat

Department of Pharmacology, Sahyadri College of Pharmacy, Methwade, Tal. Sangola Dist. Solapur-413 307, M.S., India.

ABSTRACT

Objectives: To evaluate antitumor activity potential of viper venom photoproducts SV_1 and SV_2 against Ehrlich ascites carcinoma in mice. **Materials and Methods:** Viper venom photoproducts SV_1 (*Vipera russelli*) and SV_2 (*Echis carinatus*) were generated by exposure of the venoms to UV sensitized methylene blue for 15 min. and 90 min. at 37°C respectively. The Antitumor activity of SV_1 and SV_2 at two concentrations ($1/10^{th}$ and $1/20^{th}$ dilutions) was evaluated on Ehrlich Ascites Carcinoma (EAC) inoculated to Swiss male mice. The antitumor response was measured from tumor volume (TV), packed cell volume (PCV), body weight (BW), hematological parameters (Hb, RBC, total WBC) and biochemical parameters (serum LDH, ALT, AST and hepatic tissue total protein, reduced GSH and TBAR (MDA). Half of the animals (n = 6) were used to determine the mean survival time (MST) and the percentage increase in the life span (%ILS). **Results:** The SV_1 and SV_2 decreased dose dependently TV, PCV, BW, WBC, serum levels of LDH, ALT; increased dose dependently serum AST level, hepatic total protein, reduced GSH, TBARs (MDA) and serum Hb in SV_{2A}. The results were statistically significant. The % ILS at the $1/10^{th}$ dilution of SV_1 and SV_2 was 40.27% and 29.52% respectively. **Conclusion:** SV_1 and SV_2 at $1/10^{th}$ dose level showed significant antitumor activity based on percent increase in the survival time, reduction in the tumor volume, packed cell volume and restoration of hematological and biochemical parameters towards normal value.

Key words: Non-herbal, *Vipera russelli, Echis carinatus,* Methylene blue, Ehrlich Ascites Carcinoma.

INTRODUCTION

Cancer is a malignant neoplasia consisting of group of disease due to unregulated cell growth. The world wide prevalence of Cancer is about 13% with 8.2 million death and diagnosed cases of cancer 14.1 million in 2012.[1] Presently, cancer is treated with chemotherapeutic agents, radiation therapy and surgery. Plant derived natural products such as flavonoids, steroids, alkaloids and terpenoids have been extensively investigated for the anticancer activity. However, investigation on natural non-herbal natural sources have been scanty. Since ancient times, snake venoms have been used in low concentration to treat illnesses. Snake venom is a complex mixture of substances consisting of enzymes, polypeptides, peptides and non protein constituents. Snake venom photo-products are non-lethal however biologically active components that produce wide spectrum of pharmacological activities of therapeutic significance.

Anticancer action to various venoms and venom components has been reported. Calmette and coworkers reported for the first time, an antitumor activity of snake venom on adenoma cells.[2] Anticarcinogenic action of *Naja kaothia* venom (NKV) and *Vipera russelli* venom (VRV) may be by inducing apoptosis as evidenced from membrane blebbing, chromatin condensation and fragmentation.[3] A heat stable protein purified from Daboia *russelli russelli* venom 'drCT-1' [(7.2kDa), 125 µg/kg/i.p./d X10d)], decreased significantly EAC cell count and increased the survival time of tumor bearing

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Correspondence Address
Dr. Shivaji P. Gawade
Principal, Sahyadri College of Pharmacy,
Methwade Road, Tal. Sangola, Dist. Solapur,
M.S.-413 307, India.
Mobile: 9096227261
E-mail: gneskw@gmail.com

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mice (178.6%),** in comparison with untreated tumor bearing control.[4] Venom of *Echis calotarus* significantly decreased EAC cell count and increased significantly survival time of the venom treated tumor bearing mice (52.3%)* in comparison with untreated tumor bearing control.[5]

The photooxidised *Vipera russelli* (SV_1) and *Echis carinatus* (SV_2) venom products are promising natural nonherbal therapeutic alternatives (NNTA) under extensive preclinical evaluation for its sedative, analgesic, anti-inflammatory, coagulant and cardiac stimulant.[6,7] and antidepressant, antidementia properties[8] respectively. In the present paper, viper venom photo products SV_1 and SV_2 were evaluated for the antitumor potential at $1/10^{th}$ and $1/20^{th}$ dilutions against Ehrlich ascites carcinoma for tumor growth response, hematological, biochemical parameters and survival studies in mice.[9,10]

MATERIALS AND METHODS

Viper snake venoms: *Vipera russelli* and *Echis carinatus* purchased from Haffkine Institute, Mumbai in lyophillised powder form.

Experimental animals: The female Swiss albino mice were purchased from SKN College of Pharmacy, Kondawa, Pune, housed and maintained under standard laboratory conditions at temp. $25^0 \pm 2^0$C with dark/light cycle (14/10). Animals were allowed free access of pellet diet (Pranav Agro Industries, Sangli) and water *ad libitum*. The animal, care and handling were carried out in accordance with the guidelines of CPCSEA (1314/ac/09/CPCSEA dt.21.12.2009). The experimental protocols were approved by the institutional animal ethical committee (SCOP/IAEC/020,021/11-12).

Ehrlich ascites carcinoma cell culture: Ehrlich ascites carcinoma (EAC) is a liquid form of ascites carcinoma in peritoneum of mouse. EAC *in vivo* cell culture maintained in mice was procured from Dept. of Pharmacology, HSK College of Pharmacy, Bagalkot, Karnataka. EAC cells grow in suspension in the peritoneal cavity of mice and in 4 or 6 days after passage, the ascetic fluid is formed.[11,12] Ascetic fluid is a gray white or occasionally light bloody viscous liquid which provide nutrition to tumor cells for tumor growth. It contains 10 million neoplastic cells in 0.1 ml.

Photochemical method: The method of photo oxidation used earlier[13] was closely followed with modifications to suit our laboratory conditions to generate photooxidised venom products SV_1[9] and SV_2.[10] The flow chart of photochemical method for SV_1 is summarized as follows

Flow chart of photochemical method

600 µg/ml (2 ml): Venom sample of *Vipera russelli*

[The venom concentration is based on the lowest concentration of venom that causes 100 % mortality in mice.] + methylene blue in phosphate buffer (0.05 M, pH 6.8) (2 ml)

Exposure to UV light (UV lamp Phillips, Holland, TUV 15 W, G15J8)

Distance of 5 cm at temperature 37^0C $\pm$ 1^0 C.

Gentle stirring (15 min.)

Addition of activated charcoal (200 µl of 1% w/v)

Stirring (5 min.)

Filtration (0.2 µ) syringe filtration unit

Absorbance measurements (280 nm on UV/VIS spectrophotometer (Shimadzu, Model no. 1601).

Safety studies in mice: The venom samples were injected intraperitoneally with decreasing concentration from 60 µg to 30 µg per 20 g of mouse. The lowest concentration producing death of all three mice is interpreted as a minimum lethal dose. The equivalent doses of snake venom photo-products SV_1 and SV_2 were injected intraperitoneally and animals were observed for mortality and behavioral symptoms for 7 days.

Viability of EAC cells

EAC cell line was maintained in our laboratory by i.p. transplantation of 0.2 ml of $2x10^6$ cells/mouse after every 10 days. Viability was determined using Trypan blue exclusion assay. The cells were stained with 0.4% trypan blue in normal saline. Percent viability was determined from the ratio of unstained cells to the total cells.[12]

Treatment protocol

The EAC cells were collected from the donor mice. The EAC cell count was adjusted to $2x10^6$ cells/ml using sterile isotonic saline. Swiss albino mice were grouped into four groups of twelve mice each. All groups of mice were inoculated with 0.2 ml of EAC cells intraperitoneally except normal group on day '0'. On first day, 5 ml/kg of 0.9% NaCl was administered orally in group I. Group II was EAC control whereas Gr III received standard 5 Fluorouracil (5 FU) intraperitoneally for 14 days. SV_{1A} & SV_{1B} (3.25 µg/20g & 1.625 µg/20g) and SV_{2A} & SV_{2B} (15µg/20g & 7.5 µg/20g) in two different concentrations were administered intraperitoneally on day 1^{st} and day 8^{th} in group IV (SV_{1A}), group V (SV_{1B}) and group VI (SV_{2A}), group VII (SV_{2B}) respectively. Six mice from each group were sacrificed on day 15^{th} to study antitumor activity, hematological and hepatic

biochemical parameters. Remaining six animals from each group were maintained on feed and water *ad libitum* to evaluate percent increase in life span with reference to the EAC normal group.[12, 14]

Antitumor activity studies

The antitumor activity of SV_1 and SV_2 was evaluated from the reduction body weight, ascites tumor volume, packed cell volume. Half of the animals were used to determine the mean survival time (MST) and percent increase in the life span (%ILS). Ascetic fluid volume was measured by collecting fluid in a graduated centrifuge tube. The packed cell volume was determined by centrifuging ascetic tumor volume at 1000 rpm for 5 minutes.

Mean survival time (MST)

Six mice from each group were observed daily for the mortality. MST is given by equation.

$$MST = \frac{\text{Mean of survival time in days of each mice in a group}}{\text{Total number of mice in a group}}$$

Percentage increase in the life span (% ILS)

Percentage increase in the life span is given by ratio.

$$\%\ ILS = \frac{\text{Mean survival of treated group}}{\text{Mean survival of control group}} \times 100$$

Hematological parameters

Blood samples from mice were collected in capillary from retro orbital plexus for hematological parameters such as hemoglobin, RBC and WBC count. The measurements of all hematological parameters were performed by analyser (Mode: 3C3000 Plus, *Mindray*).

Hepatic and Serum biochemical parameters

The mice after blood sample collection were anesthetized and operated to expose the liver. The liver was perfused with saline before excision, rinsed with ice cold saline followed by cold 0.15 M Tris HCl (pH 7.4), blotted dry and weighed. A 10% w/v homogenate was prepared in 0.15 M Tris HCl buffer. It was centrifuged at 1500 rpm for 15 minutes at 4^0C. The supernatant was used for the estimation of biochemical parameters such as total protein, reduced glutathione and TBARS (MDA). Serum samples were used for the estimation of Lactate Dehydrogenase, (LDH) Aspartate Aminotransferease (AST) and Alanine Aminotransferase, (ALT) levels.

Estimation of Total protein

Protein sample was allowed to react with copper in alkaline medium. The copper treated protein reduces Folin Ciocalteau reagent to impart maximum color at pH 10. The liver homogenate was mixed with 90% alcohol in 1:10 proportion. The resulting precipitate was dissolved in 1 ml of 0.1N NaOH and used for the estimation of total protein according to the Lowry's method.[15]

Estimation of Serum enzymes

Lactate dehydrogenase (LDH), Aspartate aminotransferase (AST) and alanine aminotransferase (ALT) were estimated using kits of Span Diagnostics Ltd.Surat, Gujarat. The kinetic factor (K) of the reaction was calculated by using following equation.

$$K = (1/M') \times (TV/SV') \times (1/P)x10^6$$

Where TV = sample volume + working reagent volume. SV' = sample volume; M' = Molar extinction coefficient of NADH = $6.22x10^3$ lit/mol/cm at 340 nm, P = optical path length; 10^6 = constant. The enzyme activity was measured in terms of IU/L = ΔA/minute x kinetic factor.

Serum Lactate Dehydrogenase

LDH catalyse the transformation of pyruvate to lactate with simultaneous oxidation of reduced NADH to NAD^+. The rate of decrease in the absorbance due to the formation of NAD is proportional to the LDH activity.

The LDH activity was measured at 340 nm and estimated using equation.

LDH activity (IU/L) = ΔA/minute x kinetic factor (K)] where K = 16030.

Serum Aspartate Aminotransaminase

AST catalyse the transformation of l-aspartate and α-ketoglutarate to oxaloacetate and l-glutamate. During further transformation of oxaloacetate to l-malate, NADH is oxidized to NAD^+. The rate of oxidation of NADH to NAD^+ was measured at 340 nm.

The AST activity was estimated using equation,

AST activity (IU/L) = [A/minute x kinetic factor (K)] where K = 1768.

Serum Alanine Aminotransaminase

ALT catalyse the transformation of L-Alanine and (α-ketoglutarate to pyruvate and l-glutamate. During further transformation of pyruvate to lactate, NADH is oxidised to NAD^+. The rate of oxidation of NADH to NAD^+ was measured at 340 nm.

The ALT activity was estimated using equation

ALT activity (IU/L) = [A/minute x kinetic factor (K)] where K = 1768.

Hepatic Tissue Enzymes Estimation

Estimation of reduced glutathione

Reduced glutathione, a natural water soluble scavenger, in the cytoplasm inhibits free radical mediated lipid peroxidation. The intensity of color developed was measured spectrophometrically at 412 nm and GSH value was obtained by interpolation on graph of standard GSH verses absorbance.[16,17]

Estimation of Lipid peroxidation

The lipid peroxidation, break down the polyunsaturated fatty acids of membrane phospholipids resulting into generation of malonaldehyde (MDA) which reacts with thiobarbituric acid, forms thiobarburic acid reactive substances (TBARS), lipid peroxides and aldehyde. TBARS were expressed in terms of MDA. The MDA content of the test liver tissue was obtained from the standard curve of concentration of MDA verses absorbance at 532 nm.[18]

Statistical analysis

All values were expressed as mean ± SEM (n = 6). The EAC treated group was compared to control group using student 't' test. All treated groups were compared to EAC control group and analysed statistically using one way ANOVA followed by Dunnett test of multiple comparison. The values of $p< 0.05$ were considered to be significant.

RESULTS

Effect on Mean survival time (MST) and percent increase in life span (%ILS)

The MST was significantly prolonged in 5 FU treated EAC mice in comparison to EAC control group. The SV_1 and SV_2 prolonged MST dose dependently however, prolongation was lesser than that of 5 FU. The result of SV_1 and SV_2 on MST at two dose levels were statistically significant except for SV_{1B} and SV_{2B}. Table 1 summarised the effect on MST and percent increase in the life span.

Effect on Body weight against EAC in mice

As shown in the Table 1, the body weight as a measure of tumor growth response in mice was increased significantly in EAC mice as compared to saline control mice. The body weight of 5 FU treated EAC mice were decreased significantly. The SV_1 and SV_2 decreased body weight dose dependently at two concentrations $1/10^{th}$ and $1/20^{th}$. However the effect on body weight is lesser than that of 5FU treated EAC mice. The results were statistically significant except for SV_{2B} treated EAC mice.

Effect on Tumor volume and packed cell volume against EAC in mice

As shown in the Table 1, the TV and PCV were completely abolished in 5 FU treated EAC mice. The TV and PCV was decreased dose dependently and significantly in SV_1 and SV_2 treated EAC mice at two concentrations $1/10^{th}$ and $1/20^{th}$. The effect of SV_1 and SV_2 on TV and PCV was statistically significant except for SV_{2B} treated EAC mice.

Effect of SV_1 and SV_2 on hematological parameters against EAC mice

The results of SV_1 and SV_2 on hematological parameters in comparison with 5 FU treated EAC were summarized in Table 2. The effect of SV_1 and SV_2 on WBC count was more significant as compared to the effect on Hemoglobin and RBC count. The WBC count was significantly decreased in 5FU treated EAC mice. The WBC count was decreased dose dependently by SV_1 and SV_2 however, the effect was lesser than that of 5 FU group. The results of SV_1 and SV_2 at two dose levels were statistically significant. The hemoglobin and RBC count were increased significantly in 5FU treated EAC mice. These values were increased dose dependently in SV_1 and SV_2 treated EAC mice. However the results were statistically insignificant except SV_{2A} treated EAC mice.

Effect of SV_1 and SV_2 on biochemical parameters against EAC mice

The results of SV_1 and SV_2 on biochemical parameters of serum and hepatic tissue levels in comparison with 5 FU treated EAC mice were summarized in Table 3. The serum LDH, AST, hepatic tissue total protein and TBARS levels were decreased significantly in 5 FU treated EAC mice. The effect of SV_1 and SV_2 treated EAC mice on LDH, AST, total protein and TBARS was decreased dose dependently however the effect was lesser than that of 5 FU but statistically significant except for SV_{2B} on AST, total protein and TBARS levels. The serum ALT and hepatic reduced GSH levels were elevated significantly in 5FU treated mice in comparison with EAC control mice. The serum ALT and hepatic reduced GSH values were dose dependently increased in SV_1 and SV_2 treated EAC mice. The effect

Table 1: Effect of SV_1 and SV_2 on tumor activity against EAC in mice

Parameters	Normal	EAC	Std (5FU)	SV_{1A} + EAC	SV_{1B} + EAC	SV_{2A} + EAC	SV_{2B} + EAC
Body weight (g)	10.51 ± 2.01	*** 62.93 ± 5.09	*** 2.29 + 0.84	*** 27.32 ± 2.47	** 45.02 + 4.96	*** 32.66 ± 3.87	50.47 ± 5.09
Mean survival time (day)	---	24.83 ± 1.05	*** 60.83 + 1.91	*** 34.83 ± 0.79	28.67+ 0.84	** 32.17 ± 1.22	27.33 ± 0.99
Increase in life span (%)	---	---	144.96	40.27	15.47	29.52	10.06
Tumor volume (ml)	---	6.45 ± 0.33	0.00	*** 2.87 ± 0.12	*** 4.48 + 0.19	*** 3.4 ± 0.30	5.57 ± 0.19
Packed cell volume (ml)	---	3.13 ± 0.17	0.00	*** 1.18 ± 0.08	*** 2.17 + 0.10	*** 1.38 ± 0.17	2.57 ± 0.07

Values are expressed as Mean ± SEM (n=6)
EAC group is compared with normal control group using student't' test.
Treatment group (Std and Test drug) are compared with EAC control group using one way ANOVA post hoc Dunnett test *p<0.05,**p<0.01, ***p<0.001

Table 2 : Effect of SV_1 and SV_2 on hematological parameters against eac in mice

Sr.No.	Parameters	Normal 0.9% NaCl	EAC control	Standard 5 FU	SV_{1A}	SV_{1B}	SV_{2A}	SV_{2B}
01	Hemoglobin (g%)	13.5 ± 029	### 9.71 ± 0.01	*11.45 ± 0.26	11.15 ± 0.82	10.70 ± 0.27	** 11.28 ± 0.51	10.68 ± 0.34
	% Increase		100	17.92	14.83	10.20	16.17	9.99
02	RBC (x 10^9 ml)	8.79 ± 0.24	### 6.98 ± 0.18	** 7.79 ± 0.10	7.40 ± 0.20	7.09 ± 0.10	7.25 ± 0.22	7.06 ± 0.16
	% Increase		100	11.61	6.02	1.58	3.87	1.15
03	WBC (x 10^9 ml)	8.58 ± 0.57	### 47.45 ± 2.25	*** 11.25 ± 0.79	*** 26.42 ± 1.43	*** 38.92 ± 1.27	*** 31.18 ± 1.4	* 41.98 ± 0.88
	% decrease		100	76.29	44.32	17.98	34.29	11.53

Values are expressed as Mean ± SEM (n=6) ,
EAC group is compared to normal group using student 't' test ;
Treatment groups (Std and Test groups) are compared with EAC control group using one way ANOVA, post hoc Dunnett test [*p<0.05,**p<0.01,***p<0.001]

however lesser, than that of 5 FU but statistically significant except for SV_{1B} and SV_{2B} on hepatic reduced GSH level.

DISCUSSION

In the present paper antitumor activity of two viper venom photoproducts SV_1 and SV_2 was evaluated comparatively with reference to the standard antineoplastic drug 5-Fluorouracil against EAC bearing mice. The photo-products SV_1 and SV_2 were generated by exposure of viper venoms, *Vipera russelli* and *Echis carinatus* to UV radiation in the presence of methylene blue. The original effective concentration of photoproducts SV_1 and SV_2, diluted to $1/10^{th}$ and $1/20^{th}$ were used to evaluate its antitumor potential.

Rapid increase in ascetic fluid with tumor growth would be the means to meet the nutritional requirement of tumor cells[19]. Prolongation of the life span at $1/10^{th}$ dilution of SV_1 and SV_2 (SV_{1A} and SV_{1B}) may be by decreasing the nutritional fluid volume and the tumor growth thereby increasing the life span of EAC bearing mice. The %ILS at $1/10^{th}$ dilution of SV_1 especially showed higher antitumor activity than that of SV_2 against EAC bearing mice. The antitumor potential of SV_1 and SV_2 was evident from the significant prolongation of the survival time, significant reduction in TV and PCV against EAC bearing mice.

The decrease in Hemoglobin and RBC count in EAC bearing mice may be due to iron deficiency, hemolytic or myelopathic conditions[20]. The effect of SV_{2A} treated EAC bearing mice on hemoglobin level was statistically significant. The concentration dependent ameliorating effect of SV_1 was higher than that of SV_2 at two dose levels on WBC count and suggestive of cell protective action by restoring the leucocytes count towards normal value.

The serum AST level was significantly increased whereas serum ALT level was significantly decreased in EAC bearing mice in comparison to saline control.

Table 3 :Effect on SV_1 and SV_2 on biochemical parameters against EAC cells in mice

Sr.No.	Parameters	Normal 0.9% NaCl	EAC control	Standard 5 FU	SV_{1A}	SV_{1B}	SV_{2A}	SV_{2B}
01	AST (IU/L)	169 ± 3.49	### 320.2 ± 10.18	*** 206.4 ± 4.48	*** 246.1 + 2.32	*** 279.4 ± 2.42	*** 263.8 ± 3.06	297.9 ± 9.58
	% decrease		100	35.54	23.14	12.74	17.61	6.96
02	ALT (IU/L)	47.98 ± 1.07	### 29.92 ± 0.73	*** 39.7 ± 0.73	*** 36.45 + 0.46	*** 34.98 ± 0.47	*** 34.97 ± 0.34	* 33.08 ± 0.45
	% increase		100	32.69	21.82	16.91	18.88	10.56
03	LDH (IU/L)	1668 ± 58.24	### 5777 ± 43.64	*** 2898 ± 67.71	*** 3971 + 60.10	*** 4879 ± 60.34	*** 4514 ± 34.34	*** 5022 ± 72.63
	% decrease		100	49.84	31.26	15.54	21.86	13.07
04	Total Protein (mg/ml)	1.01 ± 0.01	### 1.24 ± 0.01	*** 0.99 ± 0.01	*** 1.00 + 0.02	** 1.15 ± 0.03	*** 1.12 ± 0.03	1.18 ± 0.02
	% increase		100	20.16	19.35	7.26	9.68	4.84
05	Reduced GSH (µg/mg tissue protein)	164.2 ± 3.79	### 64.07 ± 3.64	*** 151.3 ± 5.40	*** 120.6 + 5.24	77.83 ± 3.09	*** 88.99 ± 4.44	68.33 ± 2.83
	% increase		100	136.2	88.23	21.48	38.90	6.65
06	TBRS (nM of MDA / mg of tissue protein)	11.87 ± 0.73	### 22.65 ± 0.50	*** 12.24 ± 0.78	*** 15 ± 1.19	** 17.8 ± 0.66	*** 16.81 ± 0.51	20.42 ± 0.65
	% decrease		100	45.96	33.77	21.23	25.78	9.85

Values are expressed as Mean ± SEM (n=6), and in terms of % decrease and % increase with reference to (+) control of EAC
EAC group is compared to normal group using student 't' test ;
Treatment groups (Std and Test groups) are compared with EAC control group using one way ANOVA ,post hoc Dunnett test [*p<0.05,**p<0.01,***p<0.001]

The reduction in serum ALT level in tumor bearing mice could be due to reduced transamination of alanine to pyruvate which may be due to inhibition of gluconeogenesis in liver and kidney of tumor bearing mice, increased utilization of hepatic and free amino acids alanine and leucine for the production of hepatic and tumor cell protein.[21] The accelerated gluconeogenesis in tumor bearing mice may be due to significant decrease in the total tissue protein, significant increase in BUN, AST and LDH.[22] The serum LDH initiates tumor formation and it's metabolism. In SV_1 and SV_2 treated EAC bearing mice, significant concentration dependent decrease of LDH levels were observed. However it was lesser than that of 5 FU treated EAC bearing mice . The reduced GSH is a vital antioxidant, inhibits the process of tumor formation. The reduced GSH level was increased significantly towards normal value in SV_{1A} and SV_{2A} treated EAC bearing mice. The MDA is a free oxygen radical intermediate generated during oxidative degradation of cancerous tissue as a terminal metabolic product of lipid peroxidation.[23] The reduction in MDA level towards normal value, indicated inhibition of hepatic lipid peroxidation. The reduction in MDA level decreased cellular damage by limiting the free radical production in tumor bearing mice.

The SV_1 and SV_2 possess multiple target sites of therapeutic significance. For the first time, experiments were conducted by diluting the photoproducts to $1/10^{th}$ and $1/20^{th}$ of original effective concentration of venom photo-products.

To summarise, SV_1 and SV_2 at $1/10^{th}$ dose level revealed significant antitumor activity based on, the prolongation of percent survival time, reduction in the tumor volume ,packed cell volume and restoration of hematological and biochemical parameters towards normal value. Further studies by increasing the concentration range of SV_1 and SV_2 will substantiate significance of these NNTAs as a promising multitarget IND in the neoplastic diseases not responding to the conventional therapies.

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CONFLICT OF INTEREST

The authors declare no conflict of Interest.

ABBREVIATION USED

EAC, TV, PCV, BW, LDH, ALT, AST, MST, NKV, VRV, NNTA, 5 FU, (%ILS), BUN (blood urea nitrogen).

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