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Chloroform Extract of *Solanum trilobatum* Inhibits the Progress of Ehrlich Ascites Carcinoma in Mice

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ABSTRACT

This study was initiated to evaluate the modulating effect of chloroform extract of *Solanum trilobatum* (CST) on Ehrlich Ascites Carcinoma (EAC) in mice. *In vitro* cytotoxicity of CST on Ehrlich Ascites Cells (EAC) and Human Leukemic cells (HL-60 cells) were evaluated using trypan blue staining. To study the *in vivo* effect of CST, tumors were introduced into experimental animals by intraperitoneal injection. Animals were treated with CST (400 mg kg⁻¹ b.wt., after 24 h and 7 days of tumor inoculation for five alternate days. Cisplatin, an antitumor drug (2 mg kg⁻¹ b.wt.) was used as a positive control starting from first and the seventh day of tumor inoculation, respectively. After the experimental period, antitumor parameters (ascitic tumor volume, mean survival time and viability of tumor cells), hematological studies and biochemical parameters (activities of SGOT, SGPT, ALP and LDH) were measured. Results showed that CST strongly inhibited the growth of HL-60 and EAC cell line in *in vitro* condition. In *in vivo* conditions, administration of CST reduced the tumor volume and the viability of tumor cells as well as increased the mean survival time of EAC inoculated mice. Activities of liver enzymes were found to be restored by the administration of CST. Histopathological examination showed that CST was capable of reducing the damage caused to the liver by the excessive tumor growth. The cytotoxic and antitumor effect demonstrated by this study supported the fact that the antioxidant components present in CST may be a promising source of antitumor compounds for managing different types of cancer.

Key words: HL-60, cytotoxicity, anti tumor, chemotherapy, natural products

INTRODUCTION

Cancer continues to be one of the leading causes of death despite significant advances in medical technology for its diagnosis and treatment. The widespread place of cancers due to the aggressive, invasive and metastatic nature of cancer cells resulted chemotherapy to emerge as the preferred medical choice to surgery and radiation (Kapadia *et al.*, 2000). Unfortunately most of the chemotherapeutic agents exhibit severe toxicity and undesirable side effects. Moreover many of them used for cancer treatment are highly expensive, mutagenic, carcinogenic and teratogenic (Jagetia and Rao, 2006). Alternatively, natural products derived from plants could be used for

cancer prevention due to their inexpensive and significant effectiveness at low doses and non-toxic property. As a result, recent decades have witnessed considerable increase in the discovery of natural products helpful in cancer chemoprevention with specific action on tumor cells (Kametani *et al.*, 2007). Plant derived medicine has definitely found a role in cancer treatment as the mechanism of interaction between many phytochemicals and cancer cells has been studied extensively (Kaufman *et al.*, 1999). Since cancer is associated with dysregulation of apoptosis that leads to programmed cell death immense significance is given to plant derived anticancer drugs which act through different pathways converging ultimately into activation of apoptosis in cancer cells leading to cell cytotoxicity (Mishra *et al.*, 2011).

Solanum trilobatum, a medicinal shrub found in Southern India is mainly used for the treatment of bronchitis (Anonymous, 1972). Earlier studies using this plant in our laboratory reported the cytotoxic potential of different solvent extracts from *S. trilobatum* and "Sobatam", the partially purified component of the plant obtained from the petroleum ether extract, contained β sitosterol as the active principle and exhibited antitumor activity including DMBA-induced skin carcinogenesis (Mohan and Devi, 1996, 1997). Chemoprotective action by sobatam against cyclophosphamide induced toxicity was also reported (Mohan and Devi, 1998). Reports from other laboratories are also available which specifies the anticancer potential of *S. trilobatum*. The methanolic extract of *S. trilobatum* exhibited chemopreventive effect by modulating the antioxidant status during diethylnitrosamine induced hepatocarcinogenesis (Shahjahan *et al.*, 2005).

Based on the encouraging outcome of our studies using *S. trilobatum* as a resource of obtaining anticancer principles, research for identifying more anticancer principles was initiated by the isolation of chloroform extractable portion of *S. trilobatum* (CST). Previously we have reported that CST have strong radical scavenging effect in *in vitro* conditions (Sini and Devi, 2004). Although, studies are available stating the anticancer potential of *S. trilobatum* none of them put light on the mechanism of cancer prevention. This report can be considered as the first one that deals with the *in vitro* cytotoxicity induction of CST in EAC and Human Promyelocytic Leukemic (HL-60) cells.

MATERIALS AND METHODS

Chemicals: All solvents used were analytical grade, purchased from SISCO Research Laboratories Pvt. Ltd. (Mumbai, India). The RPMI medium 1640, Fetal Bovine Serum (FBS), L-glutamine, penicillin, streptomycin, Trypan blue solution were purchased from Sigma Chemical Co. (St. Louis, MO, USA). Cisplatin (10 mg/10 mL) was purchased from Serum Institute of India, Pune, India.

Sample preparation: Fresh plants were collected from Nagercoil forest, Tamil Nadu in June 2011 and taxonomically authenticated. The whole plant excluding roots was cut into pieces, shade dried and powdered. The powder (100 g) was extracted exhaustively in soxhlet apparatus initially with petroleum ether BP (60-80°C) followed by chloroform BP (60-62°C). The extracts were then concentrated *in vacuo* until the solvent was completely removed. The yield of petroleum ether and chloroform extract (CST) was found to be 3.33 and 1.66 g, respectively. The CST was used for further experiments.

Cytotoxic activity assay: *In vitro* cytotoxicity activity of CST was done by determining the percentage viability of EAC cells and Human promyeloblastic cells (HL-60) using the trypan blue

exclusion technique (Moldeus *et al.*, 1978). Briefly, EAC cells aspirated aseptically from the peritoneal cavity of the mice were suspended in a known volume of Hank's Balanced Salt Solution (HBSS) and the cell count was adjusted to 2×10^6 cells/mL. The HL-60 was purchased from National Centre for Cell Science (NCCS) Pune, India and maintained in RPMI-1640 containing 10% FBS supplemented with L-glutamine, penicillin (100 units/mL) and streptomycin ($100 \mu\text{g mL}^{-1}$). Exponentially growing cultures were maintained in an atmosphere of 5% CO_2 per 95% relative humidity at 37°C . The EAC and HL-60 cells (2×10^6 cells/mL) were exposed to different concentrations ($10-100 \mu\text{g mL}^{-1}$ in DMSO) of CST and incubated at 37°C for 3 h. After 3 h, the trypan blue dye exclusion test was performed to determine the percentage viability.

***In vivo* antitumor assay:** Adult male inbred Swiss mice weighing 20-30 g from the colony of our department animal house were used. The animals were grouped and housed in polyacrylic cages and maintained under standard laboratory conditions. During all the experimental period the animals were fed with standard pellet diet (Hindustan Lever, Kolkata, India) and water *ad libitum*. The study was approved by the University Animal Ethical Committee. Animals were divided into six groups of 12 animals each as follows:

- Group I: Normal Control
- Group II: EAC Control
- Group III: EAC+CST (prophylatic)
- Group IV: EAC+(7 days post inoculation)
- Group V: EAC+Cisplatin (prophylatic)
- Group VI-EAC+Cisplatin (7 days post inoculation)

On day one all the groups were injected (i.p.) with EAC cells (0.2 mL of 2×10^6 cells/mouse) except the animals in group I (Normal). A day of incubation was allowed for multiplication of the cells. On the first day onwards a total five doses of 5 mL kg^{-1} b.wt., of normal saline was administered to group I and group II (EAC control) at 24 h intervals while animals in group III were administered CST (400 mg kg^{-1} b.wt.). Group IV animals received CST (400 mg kg^{-1} b.wt.) on five alternate days starting from the seventh day of tumor inoculation. Group V and VI animals received Cisplatin, a standard antitumor drug (2 mg kg^{-1} b.wt.) starting from first and the seventh day of tumor inoculation, respectively. On day 16, half of the animals in each group were sacrificed for the study of antitumor activity, hematological, serum biochemical parameters and the remaining animals were kept for the survival time of EAC-tumor bearing hosts.

Antitumor parameters: Body weight of the animals was monitored continuously throughout the experiment. The ascitic tumor volume was determined by collecting the ascitic fluid from the peritoneal cavity of mice and taking it in a graduated centrifuge tube. Tumor packed cell volume was determined by centrifuging at 1000 rpm for 5 min. A portion of the ascitic fluid was stained with 0.4% trypan blue was used for counting the number of viable cells. Mean Survival Time (MST) of each group was monitored by recording the mortality daily for 6 weeks and percentage of Increase in Life Span (ILS) was calculated by the following formula:

$$\text{ILS (\%)} = \frac{\text{MST of treated group}}{\text{MST of control group}} - 1 \times 100$$

$$\text{MST} = (\text{Day of 1st death} + \text{Day of last death}) / 2$$

Hematological studies: Blood was collected from the animals by retro-orbital puncture and the hematological parameters such as Red Blood Cells (RBC), White Blood Cells (WBC), differential count and hemoglobin content were determined using an auto analyzer.

Biochemical assays: To determine the changes in the biochemical parameters of EAC bearing mice due to the treatment of CST, activities of Glutamate Pyruvate Transaminase (GPT), Glutamate Oxaloacetate Transaminase (GOT), lactate dehydrogenase (LDH) and serum alkaline phosphatase was estimated by the procedures described earlier by Reitman and Frankel (1957) and Kind and King (1954).

Histopathology of liver: After the experimental period, mice were anesthetized and the livers were removed and preserved in 10% formaldehyde. Dehydration and clearing of the tissues were performed. Five micrometer thickness sections were prepared and stained with hematoxylin and eosin (H/E) (Mendez and Hernandez, 2005). Stained sections were qualitatively evaluated using a digital microscope (Labomed, iVu 3000, USA). The images were analyzed using Digipro software (Germany).

Statistical analysis: Mean values \pm SD was calculated for all parameters. The statistical difference was analyzed by one-way analysis of variance (ANOVA) using windows SPSS 10 followed by Duncan's post hoc test and significance was calculated as p-values and p-values <0.05 were regarded as significant.

RESULTS

Cytotoxic effect of CST on EAC cells *in vitro*: *Solanum trilobatum* (CST) showed significant cytotoxic effect against Ehrlich Ascites Carcinoma (EAC) and HL-60 cells in *in vitro* conditions. Various concentrations (10-100 $\mu\text{g mL}^{-1}$) of CST was tested for cytotoxicity. The IC_{50} value of CST against EAC and HL-60 cell lines were found to be 35 and 10 $\mu\text{g mL}^{-1}$, respectively (Fig. 1).

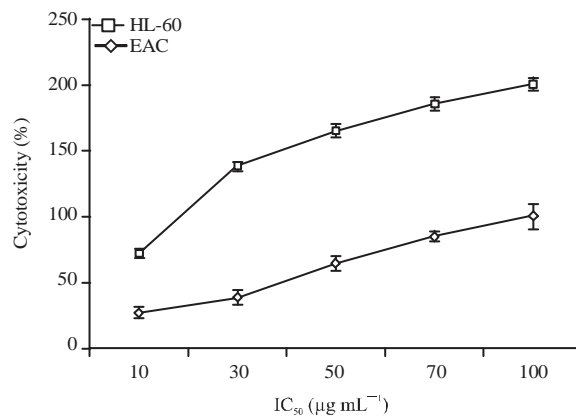


Fig. 1: Cytotoxic effect of CST on EAC and HL-60 cells, cells were stained with trypan blue and counted using a hemocytometer. Values are expressed as Mean \pm SD of three experiments

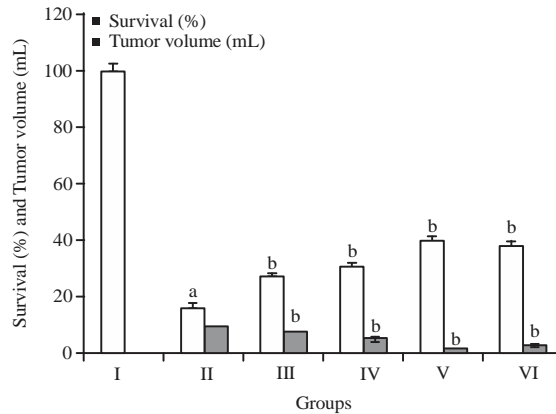


Fig. 2: Percentage survival and tumor volume in treated and control animals, Group I: Normal control, Group II: EAC control, Group III: EAC+CST (Prophylatic) (400 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group IV: EAC+CST (400 mg kg⁻¹ b.wt., from 7th day of EAC inoculation), Group V-EAC+Cisplatin (Prophylatic 2 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group VI: EAC+Cisplatin (2 mg kg⁻¹ b.wt., after 7th day of EAC inoculation). Values are mean of 6 mice±SD, ^aStatistically significant compared to Group I (p<0.05), ^bstatistically significant compared to Group II (p<0.05)

Table 1: Gain in body weight of treated and control animals

Groups	Body weight		
	Initial weight (g)	Final weight	Weight gain (%)
I	31.3±2.52	31.7±2.0	1.3±0.03
II	29.3±2.9	34.3±1.5	17.1±0.04 ^a
III	30.0±1.0	32.0±1.0	6.7±0.08 ^b
IV	29.3±1.5	30.3±2.1	3.4±0.05 ^b
V	33.7±0.6	32.2±1.0	-4.1±0.02
VI	31.3±1.2	29.33±1.5	-0.6±0.05

Group I: Normal control, Group II: EAC control, Group III: EAC+CST (Prophylatic) (400 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group IV: EAC+CST (400 mg kg⁻¹ b.wt., from 7th day of EAC inoculation), Group V: EAC+Cisplatin (Prophylatic 2 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group VI: EAC+Cisplatin (2 mg kg⁻¹ b.wt., after 7th day of EAC inoculation). Values are mean of 6 mice±SD, ^aStatistically significant compared to Group I (p<0.05), ^bStatistically significant compared to Group II (p<0.05)

Antitumor parameters

Effect on body weight gain: Ehrlich Ascites Carcinoma (EAC) tumor bearing mice showed a constant weight gain and increase in tumor volume (Fig. 2) due to cell multiplication and growth of tumor cells whereas no such increase in the body weight was found in CST administered groups, while, cisplatin treated Group V and VI showed a decrease in body weight gain much lesser compared to CST treated group (Table 1).

Effect on Mean survival Time (MST): The MST of EAC bearing mice was found to be only 16±2.0 days whereas administration of CST at a dose of 400 mg kg⁻¹ b.wt., on five alternate days starting from the first and 7th day of tumor inoculation enhanced the MST to 27±1.0 (ILS % = 62.5) and 30.67±0.58 (ILS % = 87.5) days, respectively. Administration of cisplatin at a dose of 2 mg kg⁻¹ b.wt., after 24 h of EAC inoculation significantly prolonged the MST to 40 days and percentage of ILS was 143.75 (Fig. 2).

Effect on tumor volume: The tumor volume was found to be significantly increased in EAC control animals. Both the CST administered groups exhibited significant reduction in these

parameters and the effect was more significant in group IV animals. Cisplatin treated animals in group V and VI showed significant decrease in tumor volume compared to control and CST treated animals (Fig. 2). The control of cell proliferation is crucial in maintaining cellular homeostasis and loss of this mechanism is a principal hallmark of cancer cells. Thus, the inhibition of tumor cell growth without side effects is recognized as an important target of cancer therapy. The clinical efficiency of an anticancer agent lies in its ability to inhibit the proliferation of tumors not only in early stages but also in the late stages of its development.

Effect on Packed Cell Volume (PCV): Packed cell volume was found to be significantly increased in EAC control animals. Both the CST administered groups exhibited significant reduction in these parameters and the effect was more significant in group IV animals. Cisplatin treated animals in Group V and VI showed significant decrease in tumor volume compared to control and CST treated animals (Fig. 3).

Effect on the viability of tumor cell: Viability of tumor cells were found to be significantly increased in EAC control animals. Both the CST administered groups exhibited significant reduction in these parameters and the effect was more significant in Group IV animals. Cisplatin treated animals in Group V and VI showed significant decrease in tumor cell viability compared to control and CST treated animals (Fig. 4).

Hematological studies: Hematological parameters of EAC bearing mice on day 16 was found to be significantly altered compared to the normal group. There was a decrease in Hb, RBC and lymphocytes in control mice, accompanied by an increase in WBC while CST (400 mg kg⁻¹ b.wt.,) treatment after 24 h and 7th day of tumor inoculation changed those altered parameters significantly to more or less normal value (Table 2). Cisplatin, the standard anticancer drug included in the study for comparison when administered 24 h after tumor inoculation significantly reversed the altered hematological parameters to normal values in comparison with EAC control mice. In cancer therapy the major problems encountered are of myelosuppression and anemia (Price and Greenfield, 1958). The anemia encountered in tumor bearing mice is mainly due to reduction in RBC or Hb production and this may occur either due to Fe deficiency or due to

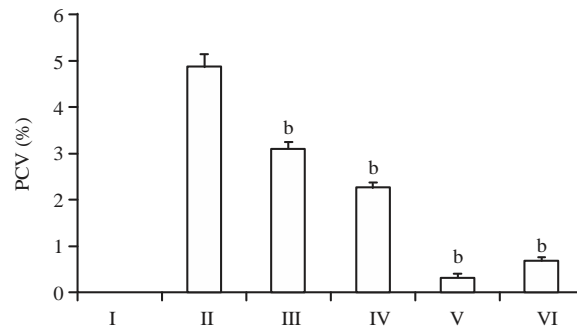


Fig. 3: PCV of treated and control animals, Group I: Normal control, Group II: EAC control, Group III: EAC+CST (Prophylactic) (400 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group IV: EAC+CST (400 mg kg⁻¹ b.wt., from 7th day of EAC inoculation), Group V: EAC+Cisplatin (Prophylactic 2 mg kg⁻¹ b.wt., body wt after 24 h of EAC inoculation), Group VI: EAC+Cisplatin (2 mg kg⁻¹ b.wt., after 7th day of EAC inoculation). Values are mean of 6 mice±SD, ^bp<0.05-statistically significant compared to group II

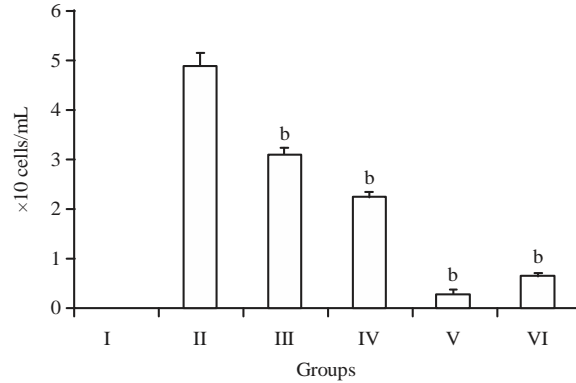


Fig. 4: Effect of CST and cisplatin on viability of tumor cells, Group I: Normal control, Group II: EAC control, Group III: EAC+CST (Prophylactic) (400 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group IV: EAC+CST (400 mg kg⁻¹ b.wt., from 7th day of EAC inoculation), Group V: EAC+Cisplatin (Prophylactic 2 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group VI: EAC+Cisplatin (2 mg kg⁻¹ b.wt., after 7th day of EAC inoculation). Values are mean of 6 mice±SD, ^bp<0.05-statistically significant compared to Group II

Table 2: Effect of CST on Hb, RBC and WBC of treated and control animals

Groups	Hb (g %)	RBC (million mm ⁻³)	WBC (10 ³ millioncells mm ⁻³)
I	13.10±0.66	5.70±0.30	8.10±0.32
II	11.00±0.33 ^a	3.90±0.13 ^a	15.10±0.91 ^a
III	12.20±0.43 ^b	4.50±0.18 ^b	10.80±0.59 ^b
IV	12.90±0.52 ^b	4.71±0.21 ^b	8.85±0.43 ^b
V	12.70±0.47 ^b	4.90±0.24 ^b	8.01±0.28 ^b
VI	10.80±0.27	4.30±0.16 ^b	9.01±0.45 ^b

Group I: Normal control, Group II: EAC control, Group III: EAC+CST (Prophylactic) (400 mg kg⁻¹ b. wt., after 24 h of EAC inoculation), Group IV: EAC+CST (400 mg kg⁻¹ b. wt., from 7th day of EAC inoculation), Group V: EAC+Cisplatin (Prophylactic 2 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group VI: EAC+Cisplatin (2 mg kg⁻¹ b. wt., after 7th day of EAC inoculation). Values are mean of 6 mice±SD, ^aStatistically significant compared to Group I (p<0.05), ^bstatistically significant compared to Group II (p<0.05)

Table 3: Effect of CST on the differential count of treated and control animals

Differential count	Lymphocytes (%)	Neutrophils (%)	Monocytes (%)
I	65.8±3.62	26.70±0.80	2.10±1.50
II	31.50±0.95 ^a	65.20±3.91 ^a	1.70±0.60
III	48.6±1.70 ^b	50.10±2.76 ^b	1.60±0.80
IV	54.7±2.50 ^b	44.10±2.21 ^b	1.80±1.30
V	62.30±3.12 ^b	31.00±1.24 ^b	1.80±0.36
VI	59.80±2.69 ^b	35.80±1.61 ^b	2.00±0.01

Group I: Normal control, Group II: EAC control, Group III: EAC+CST (Prophylactic)400 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group IV: EAC+CST (400 mg kg⁻¹ b. wt., from 7th day of EAC inoculation), Group V: EAC+Cisplatin (Prophylactic 2 mg mg kg⁻¹ b. wt., after 24 h of EAC inoculation), Group VI: EAC+Cisplatin (2 mg kg⁻¹ b.wt., after 7th day of EAC inoculation). Values are mean of 6mice±SD, ^aStatistically significant compared to Group I (p<0.05), ^bStatistically significant compared to Group II (p<0.05)

hemolytic or myelopathic conditions (Hoagland, 1982). Treatment with CST brought back the Hb content, RBC and WBC count more or less normal levels which indicates that CST possess protective action on the hemopoietic system. Differential count of treated and control animals showed that the levels of lymphocytes and monocytes were significantly reduced, while neutrophil levels were significantly increased in EAC inoculated mice. The CST treatment in prophylactic and post treatment mode showed a significant beneficial modulation of these parameters. Cisplatin treatment also showed similar pattern of modulation of these parameters (Table 3).

Table 4: Effect on serum GOT, GPT, ALP and LDH of control and treated mice

Groups	SGOT	SGPT	ALP	LDH
I	40.20±1.21	63.50±1.90	12.10±0.30	1738.00±52.14
II	128.64±8.36 ^a	165.10±11.56 ^a	42.45±2.55 ^a	2920.60±204.40 ^a
III	93.80±8.36 ^b	102.70±6.16 ^b	31.00±1.24 ^b	2299.00±164.59 ^b
IV	60.20±3.01 ^b	89.72±4.45 ^b	29.56±1.03 ^b	1997.00±99.85 ^b
V	45.60±1.60 ^b	83.70±3.77 ^b	38.31±1.92 ^b	1838.00±73.52 ^b
VI	56.40±2.54 ^b	56.40±2.54 ^b	36.43±1.64 ^b	1961.00±88.25 ^b

Group I: Normal control, Group II: EAC control, Group III: EAC+CST (Prophylatic) (400 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group IV: EAC+CST (400 mg kg⁻¹ b. wt., from 7th day of EAC inoculation), Group V: EAC+Cisplatin (Prophylatic 2 mg kg⁻¹ b.wt., after 24 h of EAC inoculation), Group VI: EAC+Cisplatin (2 mg kg⁻¹ b.wt., after 7th day of EAC inoculation). Values are mean of 6 mice±SD, ^aStatistically significant compared to Group I (p<0.05), ^bStatistically significant compared to Group II (p<0.05)

Effect on liver enzymes: When compared with normal mice, EAC bearing mice showed significant higher activity of serum GOT, while treatment with CST and cisplatin reduced the level of this enzyme compared to EAC control (Table 4). When compared with normal mice, EAC bearing mice showed significant higher activity of serum GPT, while treatment with CST and cisplatin reduced the level of this enzyme compared to EAC control (Table 4). When compared with normal mice, EAC bearing mice showed significant higher activity of serum ALP, while treatment with CST and cisplatin reduced the level of this enzyme compared to EAC control (Table 4). When compared with normal mice, EAC bearing mice showed significant higher activity of serum LDH, while treatment with CST and cisplatin reduced the level of this enzyme compared to EAC control (Table 4).

Histopathology of the liver: Normal control animals showed regular hepatocytes with no sinusoid dilation. There was no visible inflammation or necrosis. Group II animals, which served as EAC control showed the presence of hepatocytes with vesicular nuclei and sinusoid dilation filled with blood. Malignant cells are infiltrated into the sub-capsular area. Mild kupffer cell proliferation was observed. There was no inflammation or necrosis. Group II animals, received 400 mg CST, 24 h after tumor cell transplantation showed malignant cells are infiltration into the sub-capsular area. Mild kupffer cell proliferation and inflammatory cell infiltration was observed. There was no sign of necrosis. Group IV animals, received 400 mg CS after tumor transplantation showed normal hepatocytes with slight focal inflammatory cell infiltration and mild sinusoid dilation. There was no sign of malignancy or necrosis. Group V animals, received cisplatin 24 h after tumor cell transplantation showed moderated fatty acid changes with no malignancy observed. Group VI animals, received cisplatin, 7 days after tumor transplantation showed toxic changes in the hepatocytes. Tumor cell necrosis was noted with focal inflammatory cell infiltration and mild sinusoid dilation (Fig. 5).

DISCUSSION

Chemotherapy is regarded as a major treatment for the control of advanced stages of malignancies. Medicinal plants are used in various countries in the treatment and prevention of cancer since they are good sources of effective anticancer drugs (Madhuri and Pandey, 2009; Cragg and Newman, 2005).

Ehrlich Ascites Carcinoma (EAC) can be used as a model in anti-cancer which gives accurate and reliable results (Clarkson and Burchenal, 1965; Kuttan *et al.*, 1990; Sheeja *et al.*, 1997; Ramnath *et al.*, 2002; Gupta *et al.*, 2004). The reliability this experiment test lies in its ability to determine the intrinsic property of any anticancer drug, natural or synthetic through prolongation

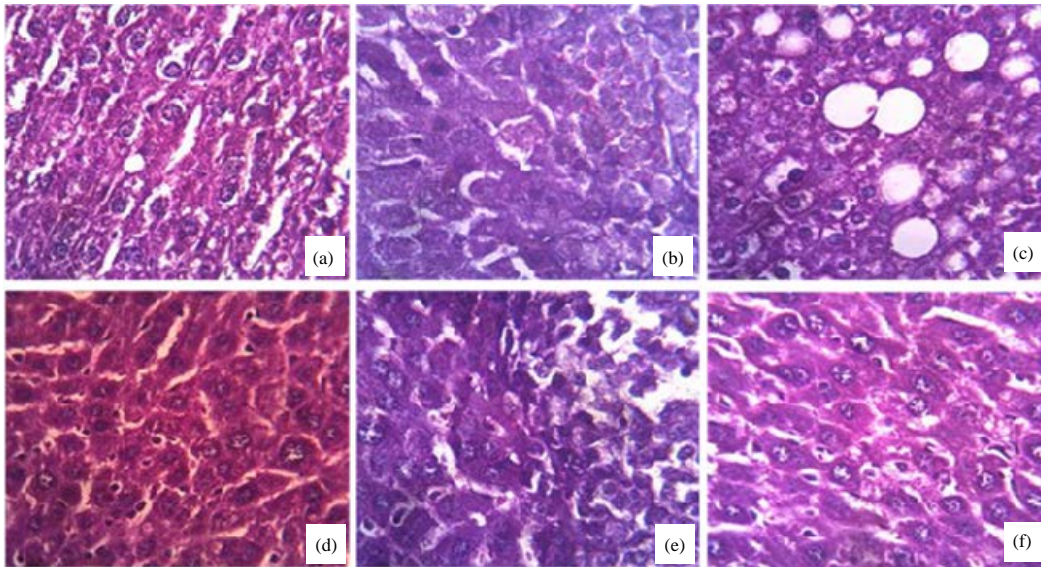


Fig. 5(a-f): Histopathology of the liver of control and treated mice bearing ascites tumor (a) Group I-Normal control, (b) Group II-EAC control, (c) Group III: EAC+CST (Prophylatic) ($400 \text{ mg kg}^{-1} \text{ b.wt.}$, after 24 h of EAC inoculation), (d) Group IV: EAC+CST ($400 \text{ mg kg}^{-1} \text{ b.wt.}$, from 7th day of EAC inoculation), (e) Group V-EAC+Cisplatin (Prophylatic) ($2 \text{ mg kg}^{-1} \text{ b.wt.}$, after 24 h of EAC inoculation) and (f) Group VI-EAC+Cisplatin ($2 \text{ mg kg}^{-1} \text{ b.wt.}$, after 7th day of EAC inoculation)

of animal life span, changes in number and viability of the cell line itself and the volume of the liquid generated by the tumor inside the peritoneal cavity (Maity *et al.*, 1999). The present study was aimed at evaluating the anti-tumour potential of chlorofom extract of *S. trilobatum* aqueous extract in EAC-bearing mice.

The CST showed significant cytotoxic activity against EAC and HL-60 cells *in vitro*. The CST showed cytotoxicity against EAC at higher concentration, while cytotoxicity against HL-60 cell line was achieved at very lower concentration. The EAC tumor bearing mice showed a constant weight gain and increase in tumor volume due to cell multiplication and growth of tumor cells whereas no such increase in the body weight was found in CST administered groups suggesting the tumor growth inhibiting property of CST. The MST of EAC bearing mice was found to be only 16 ± 2.0 days whereas administration of CST at a dose of $400 \text{ mg kg}^{-1} \text{ b.wt.}$, on five alternate days starting from the first and 7th day of tumor inoculation enhanced the MST to 27 ± 1.0 (ILS% = 62.5) and 30.67 ± 0.58 (ILS% = 87.5) days, respectively. This clearly indicates that CST has both preventive and curative effect but the curative effect was more significant. As expected administration of cisplatin at a dose of $2 \text{ mg kg}^{-1} \text{ b.wt.}$, after 24 h of EAC inoculation significantly prolonged the MST to 40 days and ILS % was 143.75.

Tumor volume of EAC control was found to be significantly very high. Treatment with CST showed a significant decrease in the tumor volume especially group IV animals received the drug several days after tumor inoculation. These results suggest that CST may be more effective in arresting the tumor growth *in vivo* at the later stages of tumor growth than the early stages. Prolongation of lifespan of the animal is considered as a greatly significant measure for evaluating

an anticancer drug (Clarkson and Burchenal, 1965). A regular and rapid increase in ascetic fluid volume is seen in EAC bearing mice and the tumor cells uses ascetic fluid as a nutritional source for tumor growth (Sundar and Nellaiah, 2013). The reduction in tumor volume and viable cell count may be due to the fact that CST treatment reduces the ascetic nutritional fluid volume and vascular permeability.

Significant elevation in the levels of SGOT and SGPT reflects the hepatocellular damages caused by a number of agents. While drug treatment reduced these elevated enzyme activities to normal levels. During malignancy the tumor marker enzymatic changes reflect overall changes in metabolism and they are increasingly appreciated as critical determinants of tumor cell behavior with sensitivity and specificity (Stefanini, 1985). The elevated level of LDH in EAC bearing mice may due to enhanced glycolysis during the growth of tumor (Nakashima *et al.*, 1988). The reduction in LDH activity on CST treatment might have controlled the glycolysis and rendered protection to membrane integrity. Alkaline phosphatase (ALP) is used as a specific tumor marker during diagnosis in the early detection of cancer (Kobayashi and Kawakubo, 1994). Serum ALP activity was found to be significantly lowered when compared to EAC control animals in both CST and Cisplatin treated animals which might be due the protective mechanism against abnormal cell growth by changing membrane permeability affecting cellular growth. Administration of CST at a dose of 400 mg kg⁻¹ b.wt., starting from the 7th day of tumor inoculation exhibited more lowering effect on serum enzymes than 24 h treatment clearly indicates the efficiency of CST to inhibit tumor development at the early as well as later stages also.

CONCLUSION

The cytotoxic and antitumor effect of chloroform extract of *S. trilobatum* (CST) demonstrated by this study supported the fact that the antioxidant polyphenols present may be a promising source of antitumor compounds deserving further screening and activity studies. The strong growth inhibition on HL-60 cells shown by CST could be due to promotion of cell apoptosis. However the active ingredients responsible for the observed effect and the specific molecular signaling pathways for the induction of apoptosis remain to be identified.

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