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In Vivo Anti-Cancer studies of *Simarouba glauca* aqueous extract leaves on Ehrlich Ascites Carcinoma model in mice

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ABSTRACT

The present study has been carried out to explore the in vivo anticancer activity of *Simarouba glauca* aqueous extract leaves against Ehrlich Ascites Carcinoma (EAC) in Swiss albino mice.The in vivo anticancer activity has been evaluated against EAC cells in Swiss albino mice by monitoring parameters including body weight, mean survival time and hematological parameters. This study indicated that aqueous extract of *S. glauca* DC leaves (500 mg/kg, p.o.) that significantly increase life span, compared to control group. 5-Flurouracil (20mg/kg, p.o.) was used as a positive control. *Simarouba glauca* DC leaves (500 mg/kg, p.o.) alerted the hematological parameters such as hemoglobin content increased, RBC increased and WBC decreases compared to cancer control. In conclusion, *S.glauca* DC leaves (500 mg/kg, p.o.) shows anticancer activity in EAC induced carcinoma in mice.

Key words: *Simarouba glauca aqueous extract leaves, Ehrlich's ascites carcinoma, Anticancer, 5-Fluorouracil.*

INTRODUCTION

Cancer remains one of the leading causes of morbidity and mortality globally. Amongst the non communicable diseases, cancer is the second leading cause of death after cardio vascular disease.[1,4] Cancer is responsible for 1 in 8 deaths worldwide. More than AIDS, Tuberculosis and Malaria together.[5]

Anticancer activity of plants has been recognized for centuries. One such plant is *Simarouba glauca*.

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Simarouba plant is very famous for prevention and cure for cancer, but in the various stages of cancer its usage will not give much result, and may even worsen the condition of cancer. *Simarouba glauca* is a medium sized tree native to Florida in South America. The common name is Paradise tree. This tree is not mentioned in *Ayurveda* text books. Sri Sri Ravishankar Ji, founder of Art of Living, gave this plant an Indian name *'Lakshmi Taru'*. The name *Lakshmi Taru* or Paradise tree is commonly agreed by most of the Indian languages.

The tree grows well in sunny regions and needs no special care. *Simarouba* leaves and leaf extracts are used traditionally by native populations in treating various ailments like fever, dysentery, cold etc. The decoction of the leaves is said to raise the natural immunity of the body so well that the patients could find themselves off from common ailments.

Simarouba is well known for its different types of pharmacological properties such as antihelmintic, antiparasitic, antidysentry, antitumour and

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antibacterial.[6] *Simarouba glauca* has very good anti-bacterial, anti-tumor properties. Hence *Simarouba* is very effective in reducing the size of tumors and secondary infections in cancer patients. It is very effective in curing cancer of first/second stages, whereas in later stages it can considerably increase the quality of life.

Quassinoids (ailanthinone, glaucarubinone, and holacanthone) found in *Simarouba* has anti-leukemic (against blood cancer) properties. Glaucarubinone has also been found to improve mitochondrial metabolism and hence improve energy levels. Early cancer screening performed by the National Cancer Institute in 1976 indicated that an alcohol extract of *Simarouba* had toxic actions against cancer cells at very low dosages (less than 20 mcg/ml). In South America, the leaves and bark of *Simarouba* are used as a powerful digestive aid. There are two varieties (red and white) of *Simarouba*. Both of them are equally potentialin treating cancer. The present study is planned to study anti-cancer activity of *S.glauca* aqueous extract leaves on (Ehrlich Ascites Carcinoma) EAC model in mice.[7]

Material and Methods

Collection of the plant material

Fresh leaves of *S.glauca* were collected from herbal garden of Sri Sri College of Ayurvedic Science and Research, Kanakapura Road, Bangalore. The leaves were washed thoroughly 2 to 3 times with water and autoclaved with distilled water and chopped in to small pieces. The cut leaves were divided in to two lots: Fresh leaves of *S.glauca* (FL) and dried leaves of *S.glauca* (DL).

Identification and authentication of the drug

The genuinity of the plants (stem part) was confirmed by Dr. Shivamanjunath, Senior scientist (Botany), Department of Dravyaguna, Sri Sri College of Ayurvedic Science and Research. The specimen sample of the herb has been preserved in Dravyaguna PG Department for future reference.

Solvent Extraction

Thoroughly washed dried leaves and fresh leaves of *S. glauca* were powdered with the help of blender, 5 gm dried leaf powder was mixed in 100 ml of distilled water. The extraction was successfully done by Soxhlet extractor for 48 hours. The solvent extracts were concentrated and reduced by rotary vacuum evaporator and preserved in air tight bottles at 5°C until further use.

Experimental Animals

Healthy female of body weight ranges 20-25g mice were selected. The animals were procured from Biogen laboratory animal facility, Bangalore. Animals were housed under standard conditions (temperature of 28 ± 2ºC and 50 ± 2% relative humidity with 12hr light / dark cycle) and provided with water adlibitum. The protocol (Ref.: IAEC/ABMRCP/2019-2020/27) of anticancer activity of S.*glauca* DC leaves aqueous extract in mice was approved by the Institutional Animal Ethical Committee (IAEC) of Acharya & BM Reddy College of Pharmacy, Bengaluru, Karnataka, study protocol was carried out as per the guidelines of CPCSEA.

Acute toxicity[8-11]

The method was adopted based on OECD-425 guidelines with AOT 425StatPgm (Version: 1.0). The limit test was performed in albino mice, at the test dose (2000 mg/kg, p.o.). The treated mice were observed as per guidelines. Subsequently the limit test was conducted for 5000 mg/kg, p.o. in albino mice. The dose was administered at the test dose 5000 mg/kg, p.o. and closely monitored and observed as per the guidelines.

Ehrlich Ascites Carcinoma (EAC) model[12-14]

Method

Adult Swiss Albino mice were inoculated with Ehrlich Ascites Carcinoma (EAC) and divided into five groups containing 12 mice in each group. Treatments were given orally at 24 hours after tumor inoculation and continued once daily for 10 days. On the 11th day, six animals from each group were anaesthetized and blood sample collected through retro-orbital plexuses. The hematological parameters like white blood cells (WBC), red blood cells (RBC), and hemoglobin (Hb) were estimated. The ascitic fluid was collected and

ISSN: 2456-3110 ORIGINAL ARTICLE Nov-Dec 2021

measured for tumor cell packed volume and viable tumor cell counts. The rest of the animals were kept to check average life span (ALS), percentage increase in life span (%ILS) and body weight measurement and antitumor parameters.

The treatment group details as follows

- **Group I**: Normal control received 0.9% normal saline orally.
- **Group II**: EAC control received 0.9% normal saline orally.
- **Group III**: EAC 1x10^6 cells treated with aqueous extract of *S.glauca* DC leaves (250 mg/kg, p.o.)
- **Group IV**: EAC 1x10^6 cells treated with 500 mg/kg of aqueous extract of *S.glauca* DC leaves extract (500 mg/kg, p.o.)
- **Group V**: EAC 1x10^6 cells treated with standard 5-Flurouracil (20mg/kg, p.o.)

All treatment was given for 10 days. The body weight and mean survival time (MST)of each group, consisting of 12 mice was noted. The antitumor efficacy of aqueous extract of *S.glauca* DC leaves was compared to that of 5-Fluorouracil. The mean survival time (MST) and percentage increases life span (%ILS) of each group was calculated by using the following equation.

MST (Days) = Total number of days survived by all animals in group/ number of animals group

% Increase in life span = (MST of treated group/MST of control group-1) × 100

RESULTS

In acute toxicity, there were no physical and behavioral changes till 14th day of observation period. Thus the data obtained from the study on single dose administration of *S.glauca* DC leaves extract (5000 mg/kg per mice, *p.o.*) administered and observed up to 14 days period did not shown in any physical and behavioralchanges motor activity, convulsion, clonic, catatonia, muscle spasm, hyperaesthesia, lacrimation, writhing, diarrhea, straubs reaction auditory response, arching and rolling, salivation, visual placing response, tail pinch response, auditory response and piloerection

In anticancer studies**,** the effect of *S.glauca* DC leaves extract 250 mg/kg and 500 mg/kg per oral significant (P<0.01) reduces the body weight and 5-Flurouracil (20mg/kg, p.o.) also reduction in the body compared to cancer control (Table 1). In hematology results, the effect of*S.glauca* DC leaves extract 250 mg/kg showed significant (P<0.05) increase in the hemoglobin, RBCs and significant (P<0.05) decrease in WBCs. *Simarouba glauca* DC leaves extract 500 mg/kg and 5-Flurouracil (20mg/kg, p.o.) exhibited significant (P<0.01) increase in the hemoglobin, RBCs and significant (P<0.01) decreases in WBCs (Table 2). In survival time, *S.glauca* DC leaves extract 250 mg/kg showed significant (P<0.05) increase of the life span. *Simarouba glauca* DC leaves extract 500 mg/kg and 5-Flurouracil (20mg/kg, p.o.) exhibited significant (P<0.01) increase of life span compared to cancer control (Table 3).

Table 1: Effect of *Simarouba glauca* DC leaves extract on body weight in EAC-bearing mice.

Groups	Changes in body weight (g)			
	0 day	7 days	14 days	21 days
G-I: Normal control (Saline, 5 ml/kg p.o.)	25.2 ± 2.14**	26.8 ± 1.5**	28.2 ± 1.58**	29.2 ± 2.2***
G-II: EAC control	36.7 ± 0.52	38.5 ± 0.41	40.2 ± 0.6	(Death)
G-III: *Simarouba glauca* DC leaves (250 mg/kg, p.o.)	36.5 ± 2.14	36.10 ± 1.42	35.2 ± 1.58*	33.6 ± 2.15***
G-IV: Simarouba glauca DC leaves (500 mg/kg, p.o.)	37.5 ± 1.46	36.7 ± 1.58	34.6 ± 1.52*	31.42 ± 2.32***
G-V: 5-Flurouracil (20mg/kg, p.o.)	36.4 ± 1.52	35.2 ± 1.50*	32.4 ± 2.02**	30.8 ± 2.21***

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett multiple comparison test. **P<0.5,*

ISSN: 2456-3110 ORIGINAL ARTICLE Nov-Dec 2021

P<0.1,P<0.01,* when compared to diabetic control group.

Table 2: Effect of *Simarouba glauca* DC leaves extract on hematological parameters in EAC-bearing mice.

Groups	Hematology parameters		
	Hb content (g%)	RBC (cells x10^6/mm^3)	WBC (cells x10^3/mm^3)
G-I: Normal control (Saline, 5 ml/kg p.o.)	11.29±0.67	5.15±0.46	8.45±0.28
G-II: EAC control	4.48±0.23	2.38±0.29	26.35±1.30
G-III: *Simarouba glauca* DC leaves (250 mg/kg, p.o.)	7.22±0.24*	3.74±0.36*	15.52±1.27*
G-IV: Simarouba glauca DC leaves (500 mg/kg, p.o.)	9.65±0.36**	4.31±0.42**	11.39±0.31**
G-V: 5-Flurouracil (20mg/kg, p.o.)	10.38±0.59**	4.88±0.27 **	9.76±1.35**

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett multiple comparison test. **P<0.5, **P<0.1,* when compared to diabetic control group.

Table 3: Effect of *Simarouba glauca* DC leaves extract on Mean Survival Time (MST) and percentage increase in life span (% ILS) in EAC-bearing mice.

Groups	Mean Survival Time in Days (MST)	Percentage increase in life span (% ILS)
G-I: Normal control (Saline, 5 ml/kg p.o.)	Alive	Alive
G-II: EAC control	14.32±0.32	0.00±0.0
G-III: *Simarouba glauca* DC leaves (250 mg/kg, p.o.)	20.5±0.36*	72.48
G-IV: Simarouba glauca DC leaves (500 mg/kg, p.o.)	22.4±0.25**	80.52
G-V: 5-Flurouracil (20mg/kg, p.o.)	23.2±0.62**	86.43

All the values were expressed in Mean ± SEM (n=6). The statistical analysis was carried out using one way ANOVA. Significant after analysis of variance (ANOVA) followed by Dunnett multiple comparison test. **P<0.5, **P<0.1,* when compared to diabetic control group.

DISCUSSION

Cancer disease is abnormal uncontrolled cell proliferation even after cessation of growth hormones. Now a days anticancer agents are used for treatment of cancer but complete therapy is not achieved. As anticancer agent, the effectiveness of *S.glauca* DC leaves has been showed by measuring the inhibition of cell growth, breakdown of cancer cells, reduction in tumor weight and restoring of hematological and biochemical parameters of the EAC cell bearing mice.[15] The present study determines acute toxicity of *S.glauca* DC leaves extract orally administered in mice. The toxicity level was assessed for 14 days as per the 425-OECD guidelines. Dose (5000mg/kg) was given once orally to each mice and observed for 14 days. Clinical observations like general appearance, cage side behavior including increased or decreased motor activity, convulsions, straub reaction, catatonia, muscle spasm, spasticity, opisthotonus, hyperesthesia, muscle relaxation, anesthesia, arching and rolling, lacrimation, salivation, diarrheal, writhing movement, mode of respiration and changes in skin colour was assessed with mean body weight and any mortality finding at the end of 14 days. In the present anticancer study, *S.glauca* DC proves that the number of cell growth decreased and number of apoptotic cells increased significantly at different doses 250 and 500 mg/kg per oral. All these are measured are very important aspects in justifying the effectiveness of a compound in cancer chemotherapy.[16] Quassinoids

ISSN: 2456-3110 ORIGINAL ARTICLE Nov-Dec 2021

have demonstrated positive anti-tumor activities, the bitter values of the plant family of Simaroubaceae.[17] Further study is needed in terms of molecular aspects to prove the anticancer activity.

Conclusion

Simarouba glauca DC leaves extract did not produce any mortality at 5000 mg/kg per oral dose and did not produce any observable toxic effect, no significant changes in mean body weight. Based on the observation made and recorded for 14 days study, it can be concluded that the *S.glauca* DC leaves extract has no toxic potential even at the dose of 5000 mg/kg in mice per oral. *S. glauca* DC leaves extract at 500mg/kg proves anti-cancer activity by restoring the hematological parameter and increasing life span or increasing mean survival time.

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ISSN: 2456-3110 **ORIGINAL ARTICLE** Nov-Dec 2021

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