

## Anticancer Properties of Total Alkaloid fraction of *Solanum pseudocapsicum* unripe fruits

Shrishailappa Badami\*, Satish G Dhonde, Manohar Reddy SA, Vijayan P and Suresh B

J. S. S. College of Pharmacy, Rocklands, Ootacamund 643 001, Tamil Nadu, INDIA

### SUMMARY

The total alkaloid fraction of the methanolic extract of *Solanum pseudocapsicum* unripe fruits was tested for its *in-vivo* anticancer activity against Dalton's Lymphoma Ascites model in mice. The total alkaloid fraction at 5.0 and 10.0 mg/kg body weight showed significant increase in the mean survival time and the percentage increase in the life span of tumor bearing mice. The increase in the body weight was found to be less than that of the control. However, the treatment at 20 mg/kg body weight was found to be toxic and showed a decrease in the mean survival time, and body weight when compared to control mice. The antitumor activity observed may be due to the cytotoxic activity of the total alkaloid fraction.

**Key words:** Antitumor; Dalton Lymphoma Ascites; *Solanum pseudocapsicum*; Total alkaloid fraction

### INTRODUCTION

Today we are witnessing a great deal of public interest on the use of herbal remedies. In India, Nilgiris is known as a treasure house of many medicinal plants and it has its own collection of plants which are not seen in any other parts of the country. *Solanum pseudocapsicum* (family : Solanaceae) is one such plant introduced in the gardens of Simla, Mussorie, Dun Valley, The Nilgiris, India for its brightly coloured berries and has become naturalized. Its antihypertensive, antispasmodic (Dhar et al., 1973), antiviral (Van Den Berghe et al., 1978) and antimycobacterial (Mitscher et al., 1976) properties have been reported. Several steroidal alkaloids and glycosides were isolated (Hohne et al., 1970, Chakravarty et al., 1984, Gan and Lin, 1997). Earlier studies carried out in our laboratories have shown strong cytotoxic properties of the total alkaloid fraction of the methanolic extract of unripe fruits of *Solanum pseudocapsicum* against several

cancerous cell lines (Vijayan et al., 2002). Our studies also confirmed the antitumor properties of the total alkaloid fraction of methanolic extract of leaves (Badami et al., 2003). The present study was planned to investigate the *in-vivo* antitumor activity of the total alkaloid fraction of the methanolic extract of unripe fruits of *Solanum pseudocapsicum* against Dalton's Lymphoma Ascites cell lines in mice.

### MATERIALS AND METHODS

The plant material was collected from the fields of Government Arts College, Ootacamund. Authentication of the plant material was carried out by Mr. S. Rajan, Survey of Medicinal Plants and Collection Unit Government Arts College, Ootacamund (Voucher No. 7345), where voucher specimens are preserved. The dried unripe fruits (250 g) were powdered and extracted with methanol (1 l) in a Soxhlet extractor for 18 h. The extract was then concentrated to dryness under reduced pressure and controlled temperature (50-60°C) to yield a deep brown semi-solid weighing 16 g (yield 6.4%), which was preserved in a refrigerator. The total alkaloid fraction (yield 0.8 g,

\*Correspondence: Shrishailappa Badami, J. S. S. College of Pharmacy, Rocklands, Ootacamund - 643 001, Tamil Nadu, INDIA. Fax: +91-423-2442937; E-mail: shrribadami@yahoo.com

5%) was isolated following a conventional procedure (Trease and Evans, 1978).

### Animals

Healthy adult Swiss albino mice weighing 25-30 g were obtained from J.S.S. College of Pharmacy animal house, Ootacamund, India. The animal house was well ventilated and animals had  $12 \pm 1$  h day and night schedule. The animals were housed in large spacious hygienic cages during the course of the experimental period. The animals were fed with rat pellet feed supplied by M/s. Hindustan Lever Ltd., Bangalore, India and water *ad libitum*. The experiments were performed as per the recommendations of CPCSEA, Chennai.

### Cell Lines and Chemicals

DLA (Dalton's Lymphoma Ascites) tumor cells were supplied by Amala Cancer Institute, Amalanagar, Trissur, India. Sodium CMC used was of analytical grade obtained from S.D. Fine Chemicals, Pvt. Ltd., Mumbai, India. Methotrexate was obtained from Ranbaxy Laboratories, Punjab, India.

### Antitumor Testing

Animals were inoculated with  $1 \times 10^6$  DLA cells/mouse intraperitoneally on day 0 and the inoculated mice were divided into five groups containing six mice in each group. The total alkaloid fraction was suspended in distilled water using sodium CMC (0.3%) and administered orally to the animals with the help of an intragastric catheter. Group I was served as tumor control and received sodium CMC suspension. Group II, III and IV were treated with total alkaloid fraction of the methanolic extract of unripe fruits at 5.0, 10.0 and 20.0 mg/kg body weight orally, respectively. Group V served as a positive control and treated with methotrexate 3.4 mg/kg body weight, orally. Treatment was started 24 h after the tumor inoculation, once daily for 3 days and observations were carried out for 30 days. The animals were subjected for the analysis of mean survival time (MST), percentage increase in the life span (%ILS), body weight and hematological parameters. Statistical analysis was carried out using Student's t-test. The results were judged significant if  $p < 0.05$ .

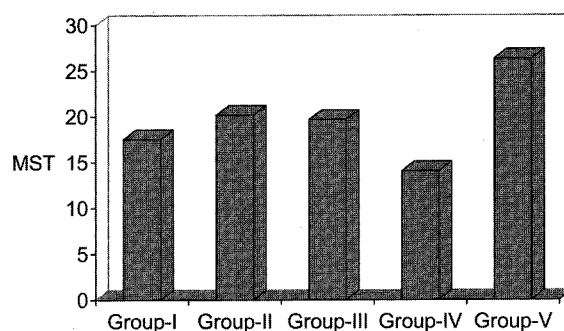


Fig. 1. Effect of the total alkaloid fraction of methanolic extract of unripe fruits of *Solanum pseudocapsicum* on mean survival time (MST) in DLA tumor bearing mice

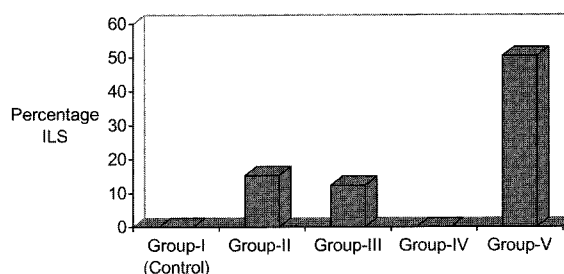


Fig. 2. Effect of the total alkaloid fraction of methanolic extract of unripe fruits of *Solanum pseudocapsicum* on %ILS in DLA tumor bearing mice

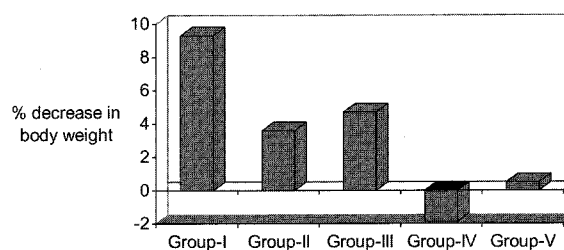


Fig. 3. Effect of the total alkaloid fraction of methanolic extract of unripe fruits of *Solanum pseudocapsicum* on average increase in body weight in DLA tumor bearing mice

## RESULTS AND DISCUSSION

In the *in vivo* anticancer studies, using DLA tumor model, the control mice survived for a period of  $17.5 \pm 0.76$  days. The mice treated for 3 days with 5.0 mg/kg body weight and 10.0 mg/kg body weight of the total alkaloid fraction of methanolic extract of unripe fruits of *Solanum pseudocapsicum* showed significant increase in the MST, when compared with control and which was  $20.16 \pm 0.87$  ( $p < 0.05$ ) and  $19.66 \pm 0.92$  ( $p < 0.05$ ) days, respectively.

The % ILS for the mice treated with 5.0 and 10.0 mg/kg body weight of the total alkaloid was found to be 15.2 and 12.34, respectively. However, the increase in the MST and %ILS of this treatment was less than that of positive control methotrexate given for the same period. The mice treated with 20.0 mg/kg body weight of total alkaloid fraction treated mice survived only for a period of  $14.0 \pm 0.58$  days. Hemorrhagic spots on the tail and palms and bleeding from the mouth and nose was observed during this treatment before 24 h of death of animals. Hence, this dose of the total alkaloid fraction is highly toxic to the animals, which was also confirmed by the decrease in their body weights.

The average increase in body weight of DLA treated control mice was found to be  $9.3 \pm 0.41$  g. The mice treated with DLA and the 5.0 and 10.0 mg/kg body weight of the total alkaloid fraction also showed an increase in the body weight, which was found to be  $3.6 \pm 0.10$  g ( $p < 0.001$ ) and  $4.7 \pm 0.14$  g ( $p < 0.001$ ), respectively. However, the average increase in body weight of these two groups of mice is more than that of standard methotrexate given for the same period (Table 1).

Hematological parameters (Table 2) of tumor bearing

mice on day 7 were found to be significantly altered from the normal group. The total WBC count was found to be increased with a reduction of the hemoglobin content of RBC. The treatment with the total alkaloid at 5.0 mg/kg body weight in tumor bearing mice caused a significant increase in the total WBC and hemoglobin content when compared with tumor control. Hence, the treatment caused a tendency of reversal towards normal values. The total number of RBC showed a modest change.

Various plants have been used in cancer therapy as a direct anticancer agents, chemotherapeutic agent, radio sensitive or immunity enhancer (Dahanukar, *et al.*, 2000). The reliable criterion for judging the value of any anticancer drug is the prolongation of life span of the animal and disappearance of Leukemic cells from blood (Lin *et al.*, 1988). In the present studies, the total alkaloid fraction of methanol extract of unripe fruits of *Solanum pseudocapsicum* at 5.0 and 10 mg/kg body weight showed a significant increase in the mean survival time and percentage increase in life span in DLA treated mice when compared with control. However, 20 mg/kg body weight dose was found to be toxic and showed a

**Table 1.** Effect of the total alkaloid fraction of methanolic extract of unripe fruits of *Solanum pseudocapsicum* on mean survival time (MST), % ILS and body weight in DLA tumor mice

Treatment	Dose (mg/kg)	Mean Survival Time (MST, days)	% Increase in Life Span (ILS)	Average increase in body wt (g)
Control (Sodium CMC, 0.3%)	---	$17.50 \pm 0.76$	---	$9.30 \pm 0.41$
Total alkaloid	5.0	$20.16 \pm 0.87^*$	15.20	$3.60 \pm 0.10^{***}$
	10.0	$19.66 \pm 0.92^*$	12.34	$4.70 \pm 0.14^{***}$
	20.0	$14.0 \pm 0.58^*$	---	$-1.90 \pm 0.15^{***}$
Standard (Methotrexate)	3.4	$26.33 \pm 0.35^{***}$	50.45	$0.5 \pm 0.02^{***}$

\* $p < 0.05$ , \*\*\* $p < 0.001$ , when compared with tumor control (n = 6)

**Table 2.** Effect of total alkaloid fraction of methanolic extract of unripe fruits of *Solanum pseudocapsicum* on haematological parameters in DLA tumor bearing mice

Treatment	Dose (mg/kg body weight)	Total count of WBC $\times 10^6$ /ml	Total count of RBC $\times 10^9$ /ml	Haemoglobin (g%)
Normal	---	$12.32 \pm 0.16$	$8.92 \pm 0.27$	$13.62 \pm 0.92$
Tumor control	---	$21.32 \pm 0.21$	$8.12 \pm 0.22$	$8.32 \pm 0.71$
Total alkaloid	5.0	$16.24 \pm 0.12^{***}$	$8.89 \pm 0.31$	$10.24 \pm 0.42^*$
	10.0	$18.34 \pm 0.16^{**}$	$8.30 \pm 0.28$	$9.82 \pm 0.68^*$
	20.0	$14.60 \pm 0.18^{***}$	$5.00 \pm 0.18^{***}$	$8.26 \pm 0.98$
Standard (Methotrexate)	3.4	$14.02 \pm 0.18^{***}$	$9.08 \pm 0.19$	$11.68 \pm 0.52^{**}$

\* $p < 0.05$ , \*\* $p < 0.01$ , \*\*\* $p < 0.001$ , when compared with tumor control (n = 6)

decrease in the MST and body weight when compared to control mice. The antitumor activity observed against DLA cells may be due to its cytotoxic effect as observed earlier (Vijayan *et al.*, 2002). There is a need to carry our the study at lower doses and for a longer duration to eliminate the toxic effects of the treatment.

Several plants belonging to Solanaceae showed strong cytotoxic and antitumor properties. Solamargine from *Solanum nigrum* (Ke, *et al.*, 1999), glycoalkaloids from *Solanum sodamaeum* (Cham *et al.*, 1987), incanumine from *Solanum incanum* (Lin *et al.*, 1990) and several other steroidal alkaloid glycosides (Mohanan and Devi, 1997; Nakamura *et al.*, 1996), have been reported to possess cytotoxic and antitumor properties. Phytochemical tests indicated that the total alkaloids of *Solanum pseudocapsicum* are steroidal in nature. Several steroidal alkaloids including Solanocapsine, Solacasine, Solacapine, episolacapine, isosolacapine and O-methyl solanocapsine have been isolated from the arboreal part of *Solanum pseudocapsicum* (Hohne *et al.*, 1970; Chakravarthy *et al.*, 1984; Gan and Lin, 1997). Hence, the observed antitumor property of the total alkaloid of *Solanum pseudocapsicum* unripe fruits may be due to the presence of steroidal alkaloids. In conclusion, the total alkaloid fraction of *Solanum pseudocapsicum* unripe fruits merit further investigation to assess its safety profile and to identify the active constituents responsible for its cytotoxic and antitumor properties.

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## REFERENCES

- Badami S, Manohara Reddy SA, Kumar EP, Vijayan P, Suresh B. (2003) Antitumor activity of total alkaloid fraction of *Solanum pseudocapsicum* leaves, *Phytother. Res.* **17**, 1001-1004.
- Chakravarty AK, Das B, Ali E, Pakrashi SC. (1984) Studies of Indian Medicinal Plants, Part 77. Structure and Stereochemistry of some new steroidal alkaloids from *Solanum pseudocapsicum* and *Solanum giganteum* by nuclear magnetic resonance spectroscopy. *J. Chem. Soc. Perk. Trans. I*, 467-474.
- Cham BE, Gilliver M, Wilson L. (1987) Antitumor effects of glycoalkaloids isolated from *Solanum sodomaem*. *Planta Med.* **53**, 34-36.
- Dahanukar SA, Kulkarni RA, Rege NN. (2000) Pharmacology of medicinal plants and natural products. *Indian J. Pharmacol.* **32**, s81-85.
- Dhar ML, Dhar MM, Dhawan BN, Mehrotra BN, Srimal RC, Tandon JS. (1973) Screening of Indian Plants for biological activity. *Indian J. Exp. Biol.* **11**, 43-54.
- Gan KH, Lin CN. (1997) Steroidal alkaloid glycoside from *Solanum pseudocapsicum*. *Chin. Pharm. J.* **49**, 315-320.
- Hohne E, Ripparger H, Schreiber K. (1970) Solanum alkaloid-XC Pontgenkristalls trukturanalyse Von N [2-Brom-benzyliden] Solanocapsin. *Tetrahedron* **26**, 3569-3577.
- Ke H, Hisayoshi K, Aijun D, Voungkui J, Shigeo I, Xinsheng Y. (1999) Antineoplastic agents 111 : steroidal glycosides from *Solanum nigrum*. *Planta Med.* **65**, 35-38.
- Lin CN, Chung MI, Gan KH. (1988) Novel antihepatotoxic Principles of *Solanum incanaum*. *Planta Med.* **54**, 222.
- Lin CN, Lu CM, Chang MK, Gan KH, Won SJ. (1990) Cytotoxic Principles of *Solanum incanum*. *J. Nat. Prod.* **53**, 513-516.
- Mitscher LH, Juvakar JV, Beal JN. (1976) Solacasine, a new steroidal alkaloid from *Solanum pseudocapsicum* possessing antimicrobial activity. *Experientia* **32**, 415-416.
- Mohanan PV, Devi KS. (1997) Effect of Sobatum on tumor development and chemically induced carcinogenesis. *Cancer Lett.* **112**, 219-223.
- Nakamura T, Komori C, Lee Y, Hashimoto F, Yahara S, Nohara T, Ejima A. (1996) Cytotoxic activities of Solanum steroidal glycosides. *Biol. Pharm. Bull.* **19**, 564-566.
- Trease GW, Evans WC. (1978) *Pharmacognosy*, 11<sup>th</sup> edn. Bailliere Tindall, London. 530.
- Van Den Berghe DA, Ieven M, Merteus F, Vlietinck AJ, Lammens E. (1978) Screening of higher plants for biological activities –II : Antiviral activity. *J. Nat. Prod.* **41**, 43-54.
- Vijayan P, Vindo Kumar S, Dhanaraj SA, Shrishailappa Badami, Suresh B. (2002) *In vitro* cytotoxicity and antitumor properties of the total alkaloids of unripe fruits of *Solanum pseudocapsicum*. *Pharm. Biol.* **40**, 456-460.