INTERNATIONAL JOURNAL OF BIOASSAYS
ISSN: 2278-778X
CODEN: IJBNHY
ORIGINAL RESEARCH ARTICLE
OPEN ACCESS

Trimodulatory effects of asoka extracts along with cisplatin and hyperthermia against sarcoma - 180 tumour bearing mice.

Varghese C. D*[1], Neenu A Santhosh[1], Hridhya M. V[1], Nair S. C[2] and Panikkar K. R[2]
[1]Post-Graduate and Research Department of Botany, St. Thomas college, Thrissur, Kerala, India.
[2]Research guide, Amala Cancer Research centre, Amalanagar, Thrissur, Kerala, India.

Received for publication: September 18, 2015; **Revised**: October 24, 2015; **Accepted**: October 27, 2015

Abstract: Cisplatin is the most active anticancer agents and have broad clinical application. *Saraca asoca*, one of the most important medicinal plants has showed potential antitumour activity and chemoprotective effects on toxicities induced by cisplatin. Purification and chemical analysis of the active compound from the bark and flower extracts of Asoka was done and observed that the plant have chemoprotective activity. The effect of administration of extracts of *Saraca asoca* flower and bark were studied in sarcoma-180 tumour bearing mice. Hyperthermia has been shown to enhance the antitumour activity of Chemotherapeutic drugs. So from the above observation, it is clear that the use of Ashoka extracts along with cisplatin and hyperthermia can enhance the antitumour activity. This trimodality treatment schedule with the Ashoka bark and flower extracts, hyperthermia and cisplatin significantly inhibited the growth of subcutaneously transplanted Sarcoma-180 solid tumours in mice.

Key words: Cisplatin; *Saraca asoka;* hyperthermia; antitumour and chemoprotective.

Introduction

Hyperthermia has been shown to enhance the antitumour activity of Chemotherapeutic drugs [1]. Surgery, radiation and chemotherapy are the conventional modalities to treat the neoplastic disease, however, many clinical trials conducted in various laboratories have revealed that elevated temperature could be an additional modality in the clinical management of cancer [2]. This approach of combining hyperthermia along with antineoplastic agents affords a measure of targeting and selective cytotoxicity unknown earlier. Cisplatin is the most active anticancer agents and have broad clinical application especially for, testicular, ovarian, head and neck, bladder and lung cancers [3]. Hyperthermia is found to potentiate the action of several antitumour agents including cisplatin [4]. However, the major limitations to the use of these drugs is their dose limiting toxicity [5]. The extract of 'Ashoka' *(Saraca asoca)* showed potential antitumour activity and chemoprotective effects on toxicities induced by cisplatin. These findings prompted us to investigate more extensively the combined effect of using Ashoka extract and hyperthermia (42^0c) along with cisplatin both in vitro and in vivo.

Materials and Methods

Ashoka extracts (bark and flower) was obtained and purified. Cisplatin was purchased from Biochem Pharmaceuticals, Bombay and were stored at 4^0c.

Thermo couple: (IT-21) was gifted by Seven-Seas Engineers, Bombay.

Tumour: Sarcoma-180 (S-180) tumour was obtained from Cancer Research Institute, Bombay.

Mice: Inbreed male Swiss albino weighing 18-20 g were used.

Determination of combined effect of *Saraca asoca* (Ashoka) extracts and hyperthermia along with cisplatin:

S-180 cells were aspirated from Swiss albino tumour-bearing mice and washed thrice with sterile normal saline. $5x10^4$ cells were added to culture bottles containing 5 ml MEM with 10% FCS. Aliquots of the drug were added one hour after the addition of the cells. The controls did not contain the drugs. Two sets of experiments were conducted. One set of culture bottles were incubated at 37^0c and another set at 42^0c for hyperthermia studies for twenty-four hours in 5% CO_2 atmosphere. After the incubation, the cells were harvested by centrifugation (1200 rpm), and counted. All the experiments were done in duplicates.

Tumour Inoculation:

All groups of mice were injected subcutaneously with $1x10^6$ Sarcoma-180 tumour cells on the right hind leg and randomized into two mice per cage.

Localised Tumour Hyperthermia:

Seven days after the tumour transplantation, mice were anaesthetized with pento barbital and the tumour limb extended. Mice were placed on rack and only the tumour bearing limb was immersed into $42.5+0.1^0$c water bath. The body of the mouse was placed on a slanted Plexiglas so that it remained out of water. The tumour limb was immersed in the water bath maintained at 42.5+ 0.1^0c for 30 minutes. A type IT-21 thermocouple microprobe was passed completely through the tumour, exiting into the water bath. The probe was

***Corresponding Author:**
Dr. George Davis,
Post-Graduate and Research Department of Botany,
St. Thomas college,
Thrissur, Kerala, India..

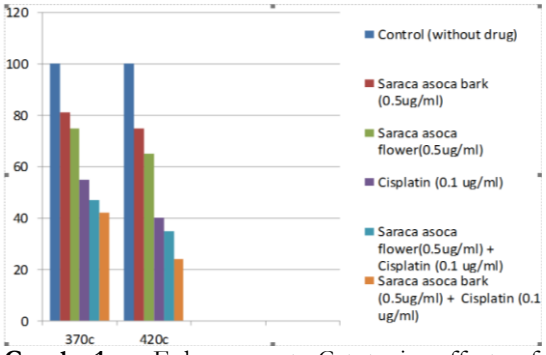
then slowly pulled through the tumour while continuous measurements were made[6]. The temperature at the site of tumour remained uniform and entirely homogenous.

Ashoka extract treatments in combination with cisplatin and Hyperthermia (*in vivo*):
Cisplatin was dissolved in sterile distilled water. The extract of 'Ashoka' bark or flower was reconstituted in 200µl of sterile saline. Drug treatment and hyperthermia commenced on the 7th day after tumour inoculation, observed on alternate days ie. (7th, 9th, 11th, 13th) and hyperthermia subsequently. The sixth group was given Ashoka flower and cisplatin as above without hyperthermia. Group seven and eight were the controls which received sterile saline (100µl) and hyperthermia and sterile saline respectively.

Determination of Biochemical and haematological studies
Peripheral blood for various studies was collected from the caudal vein at different intervals. The total leucocyte counts were performed using a haemocytometer. Blood Urea Nitrogen (BUN) Serum alkaline phosphatase (SAKP) and Serum glutamate pyruvate transaminase (SGPT) were estimated.

Results and Discussion

Effect of hyperthermia and Ashoka extracts along with Cisplatin on S-180 tumour cells (in vitro): - In presence of 0.1µl/ml of cisplatin 56 +6.9% cells survived at 37^{0}c. While at 42^{0}c the cell survival was 39.5+7.5%. 'Ashoka' bark extract alone at a concentration of 0.5µg/ml showed cell survival of 76.4+10.5 at 37^{0}c and 64+11.12% at 42^{0}c. However, the combination of cisplatin (0.1µg/ml) and Ashoka bark extract (0.5µg/ml) reduced cell survival to 44.3 +8.2% (37^{0}c) and 24.12+4.2% at 42^{0}c.



Graph 1: Enhancement Cytotoxic effect of Cisplatin in presence of *Saraca asoca* bark and flower extract and on hyperthermia (420^{0}c) to S-180 tumour cells in vitro.

In the case of flower extract alone at a concentration of 0.5µg/ml showed a cell survival of 82.4+9.8% at 37^{0}c and 70+8.5% at 42^{0}c.But the combination of cisplatin (0.1µg/ml) and flower extract (0.5µg/ml) reduced cell survival to 48.2+8.4% (37^{0}c) and 34.22+6.8% at 42^{0}c (Fig. 19). Similarly, in the case of cyclophosphamide (CTX) treated cells, a concentration of 1µg/ml showed cell survival of 42.98+6.25% at 37^{0}c and 35.36+3.53% at 42^{0}c.In combination ie., CTX (1µg/ml). Ashoka bark or flower extract (0.5µg/ml) the cell survival was reduced to 24.12+4.4% and 34.14+2.8% at 37^{0}c respectively (figure. 20). These studies indicate a lower cell survival when 'Ashoka' bark or flower extract is combined with cisplatin along with hyperthermia.

Effect of 'Ashoka' extracts and cisplatin on Hyperthermia.
Table depicts the inhibitory effect of 'Ashoka' extracts in combination with cisplatin and hyperthermia in seven-day old Sarcoma-180 tumour in mice. Combination treatment with cisplatin and 'Ashoka' bark or flower extract in the absence of hyperthermia restricted the tumour growth by 68% and 58%. While animals received trimodality treatment schedule consisting of 'Ashoka' bark or flower extract, cisplatin and hyperthermia effectively inhibited the growth of S-180 tumour by 93.3% and 83% respectively ($p < 0.001$).

Table 1: Effect of *Saraca asoca* bark and flower extracts on the growth of Sarcoma-180 tumours in mice treated with cisplatin and hyperthermia

Treatment	Dose Regimen	Mean tumour Weight (g)	Tumour growth inhibition (%)
S-180 + Cisplatin + Hyperthermia	3mg/kg i.p.	0.77 + 0.01	36
S-180 + Cisplatin	3mg/kg i.p.	0.823 + 0.08	31
S-180 + Cisplatin +*Saraca asoca* bark +Hyperthermia	3mg/kg +50mg/kg i.p.	0.08 + 0.01 ***	93
S-180 + Cisplatin +*Saraca asoca* bark	3mg/kg +50mg/kg i.p.	0.38 + 0.07 **	68
S-180 + Cisplatin +*Saraca asoca* flower +Hyperthermia	3mg/kg +50mg/kg i.p.	0.21 + 0.03 ***	83
S-180 + Cisplatin +*Saraca asoca* flower	3mg/kg +50mg/kg i.p.	0.50 + 0.05	58
S-180 + hyperthermia	-	1.1 + 0.23	08
S-180 Control mice (Sterile saline)	-	1.2 + 0.17	-

Tabular values represent mean + SD of seven mice/group for three separate experiments (N=21). Values significantly different from mice treated with cisplatin and hyperthermia. Tumour growth inhibition (%) was calculated with respect to S-180 control mice. *** $P < 0.001$ ** $P < 0.005$

Leucocyte counts and the haemoglobin levels were significantly reduced in mice which received cisplatin 3 mg/kg in conjugation with hyperthermia or without hyperthermia respectively. The leucocyte counts and the haemoglobin levels appeared normal in both the Saraca asoca bark and flower treated groups receiving trimodality therapy.

Serum chemistry studies indicated that the serum glutamate pyruvate transaminase (SGPT) levels were increased to almost two fold in the group treated with cisplatin and hyperthermia. The administration of *Saraca asoca* bark extract significantly protected the groups receiving trimodality therapy from a sharp rise in SGPT levels (Table-49). *Saraca asoca* flower extracts did not show this protective effect. The blood urea nitrogen levels were also predictably altered in the cisplatin treated groups receiving hyperthermia. However, the blood urea nitrogen was maintained in the near normal range in mice which received *Saraca asoca* bark or flower along with cisplatin and hyperthermia.

Table 2: Blood Urea Nitrogen and glutamine pyruvate transaminase levels of Sarcoma-180 tumour bearing mice treated with *Saraca asoca* along with cisplatin and hyperthermia

Treatment	Serum glutamate Pyruvate transaminase Levels(IU/L)		Blood Urea Nitrogen (mg/100 ml)	
	Day3	Day 14	Day 3	Day 14
S-180 + Cisplatin (3 mg/kg) + Hyperthermia	4.6 + 0.2	8.6+0.45	22.1+1.52	28.9+1.35
S-180 + Cisplatin (3 mg/kg)	4.7 + 0.4	9.1+ 0.5	24.0+1.07	29.7+2.0
S-180 + Cisplatin (3 mg/kg) +*Saraca asoca* bark (50 mg/kg) +Hyperthermia	5.2 + 1.1	5.2+0.3*	23.5+2.4	23.77+2.7*
S-180 + Cisplatin (3 mg/kg) +*Saraca asoca* bark (50 mg/kg)	6.0 + 1.0	7.7+0.65	25.6+3.1	25.3+2.3
S-180 + Cisplatin (3 mg/kg) +*Saraca asoca* flower (50 mg/kg) +Hyperthermia	5.3+ 0.6	7.6+0.77	23.4+1.5	24.72+1.2*
S-180 + Cisplatin (3 mg/kg) + *Saraca asoca* flower (50 mg/kg)	5.7 + 0.7	8.1+1.05	24.12+2.5	26.1+2.7
S-180 + Hyperthermia (Sterile saline)	4.1 + 0.2	4.1+0.32	22.7+0.6	22.9+1.2
S-180 + Control mice (Sterile saline)	4.0 + 0.5	4.0+0.25	22.1+0.8	22.4+1.9

Values represent the mean + SD of seven mice used per group for 3 separate experiments (N=21). Significantly different from mice treated with cisplatin and hyperthermia * $P < 0.05$ * $p < 0.02$ * $p < 0.001$

Conclusion

Hyperthermia and Ashoka extract if used together with cisplatin resulted in enhancement of the cytotoxicity to cultured S-180 tumour cells was observed. The trimodality treatment schedule with the Ashoka bark or flower extracts, hyperthermia and cisplatin significantly inhibited the growth of subcutaneously transplanted Sarcoma-180 solid tumours in mice. This effect indicated the ability of *Saraca asoca* (Ashoka) extracts of bark or flower and hyperthermia to improve the antitumour efficacy of cisplatin. Severe haematological toxicities like fall in haemoglobin levels and leucopenia after treatment with cisplatin were significantly protected by the administration of Ashoka bark or flower with hyperthermia. The elevation in the BUN, SGPT and SAKP levels were also prevented by trimodality treatment indicating protection against renal damage and liver necrosis. Thus these studies indicate the efficacy of the Ashoka bark and flower extracts to enhance the antitumour activity of cisplain in combination with hyperthermia.

References

1. Hahn, G. M., and Strande, D. P., (1976), J. Natl. Cancer Inst. 37:1
2. Abe, M., Hiraoka, M., Takahoshi, M., Ono, K., Nohar, H., (1982), Natl. Cancer Inst. Monogr. 61:411-414.
3. Schmalbach, T. K., and Borch, R. F., (1989), Cancer Res. 49: 6629-6633.
4. Murthy, M. S., Rao, L. N., Khandkar, J. D., and Scanlan, E. F., (1987), Cancer Res. 47:774-779.
5. Allan, S. G., Cornbleet, M. A., Warringtton, P. S., Golland, I. M., Leonard, L. F., and Smyth, J. F., (1984), Br. Med. J. 289:878-879.
6. Sukumar, S., Notario, V., Martin-Zanea, D., and Barbacid, M., (1983), Nature. 306-658.

Cite this article as:
Varghese C. D, Neenu A Santhosh, Hridhya M. V, Nair S.C and Panikkar K. R. Trimodulatory effects of asoka extracts along with cisplatin and hyperthermia against sarcoma - 180 tumour bearing mice. *International Journal of Bioassays* 5.2 (2016): 4791-4793.

Source of support: Nil
Conflict of interest: None Declared