WORLD JOURNAL OF PHARMACEUTICAL RESEARCH
SJIF Impact Factor 5.990
Volume 4, Issue 10, 1633-1640. Research Article ISSN 2277– 7105

MODULATORY EFFECTS OF ASOKA EXTRACTS AS CHEMOPROTECTORS TO OVERCOME CYCLOPHOSPHAMIDE INDUCED TOXICITY IN SARCOMA - 180 TUMOUR BEARING MICE

Varghese C. D.* and Neenu A Santhosh

Research & Postgraduate Department of Botany, St. Thomas' College (Autonomous), Thrissur P.O. Kerala (India) -680005.

Article Received on
4 Aug 2015,

Revised on 24 Aug 2015,
Accepted on 13 Sep 2015

***Correspondence for Author**
Prof. Varghese C.D.
Research & Postgraduate Department of Botany, St. Thomas' College (Autonomous), Thrissur P.O. Kerala (India) - 680005.

ABSTRACT

Saraca asoka (Asoka), one of the most legendary and sacred trees of India belonging to the family Caesalpinaceae is a universal panacea in Ayurveda. Further purification and chemical analysis of the active compound from the bark extract of Asoka showed that (-) - epicatechin was responsible for the observed chemoprotective activity. The effect of the administration of extracts of Asoka *(Saraca asoca)* flower and bark were studied in sarcoma - 180 tumour bearing mice with a chronic lethal dose of cyclophosphamide (15mg/kg body wt.). Combination therapy using the extracts of Asoka (flower or bark) along with cyclophosphamide significantly inhibited the growth of subcutaneously transplanted sarcoma-180 tumour in mice. Leukopenia and fall in haemoglobin levels were prevented, mice which received combination treatment regimen. Serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (SAKP) levels were also maintained in the near normal range in mice reviving cyclophosphamide along with flower or bark indicating protection against renal damage and liver necrosis.

KEY WORDS: Chemoprotection, Cyclophosphamide (CTX), *Saraca asoka*, (-)-Epicatechin.

1. INTRODUCTION

Combination chemotherapy has a recognized role in the cure of disseminated neoplasms.[14] Cyclophosphamide (CTX) an alkylating agent, is a broad spectrum anticancer drug which gets activated in the liver by microsomal enzyme system in the presence of oxygen and reduced NADPH.[1] Out of the resulting breakdown products, phosphor amide mustard B the

major alkylating agent, is supposed to be further activated at the site of action and converted to the proper cytostatic agent.[4,15] Nausea, vomiting, anorexia, leukopenia and hepatic toxicities were some of the side effects of cyclophosphamide administration[15] . Presently a variety of drugs like 2 MPG (mercapto propionyl glycine and WR 2721 (S-2-3-Amino propyl amino ethyl phosphorothioic acid) are employed to reduce these side effects.[4] The extracts of Saffron, *Nigella sativa*, garlic (*Allium sativum*) are reported to reduce the side effects of cyclophosphamide therapy.[5,6] *Saraca asoka* is found to have pharmacological properties like antimicrobial, anticancer, spasmogenic, antioxytic activities.[7,8] Ashokarishta, an Ayurvedic fermented polyherbal formulation with Asoka as the main ingredient is commonly used to control excessive bleeding, enhances repair of endometrium, eliminates fibroids, purify blood and manage stress.[9] In this paper, we have investigated the chemoprotective effects of asoka extracts towards cyclophosphamide induced toxicity in S-180 tumor bearing mice.

2. MATERIALS AND METHODS

Natural Products

Extraction and purification of the active compound:

The bark of *Saraca asoka* was collected after authentication, washed and dried in the shade. The extraction and purification of the bark extract was done according to the method of Middelkoop and Labadie.[10] Briefly, the powdered bark (100g) was then extracted with acetone (90%), three times and pooled. The combined acetone extract was concentrated to a small volume (25ml) and fractioned by successive extraction into ether and ethyl acetate soluble fraction. The ethyl acetate fraction (200 mg) was further purified by ascending paper chromatography on Whatman no 1 filter paper into homogenous compound with butanol/acetic acid/ water (6:1:2) as the solvent system the active spot was identified by spraying a test strip with ferric chloride potassium ferricyanide.[10] The active spot (bright yellow colour) located on paper, as monitored by cytotoxicity studies.[11] was cut and eluted with methanol and evaporated to dryness in a vaccum. The chemical analysis of the active (cytotoxic) compound (50 µg) obtained from the bark extract of *Saraca asoka* indicated it to be (-)-epicatechin.[10]

Chemicals

CTX was dissolved in sterile distilled water to a concentration of 5mg/ml. Cyclophosphamide (CTX) (Endoxan ASTA) was purchased from Khandelwal Laboratories, Bombay.

Tumor Cell

S-180 cells were obtained from Cancer Research Institute, Bombay as ascites tumour model and propagated In our laboratory, by i.p. transplantation of 1x 106 cells in swiss albino mice.

Chemoprotection Studies

The experiments were carried out with six groups of male swiss albino mice weighing about 20-22 gms and 9-11 weeks old. Each group contained 7 mice was housed in ventilated cages and fed with standard mouse feed (Lipton India) and water ad libitum. All the animals of 6 groups were i.p. transplanted with 1X106 S-180cells. The first groups of animals were given the first dose of CTX at a concentration of 50mg/kg. body weight i.p. 24 hours after the transplantation of the tumour cells. This same dose was given for four times on alternate days. The second group of animals were given *Saraca asoka* Flower extract 50mg/kg. 24 hrs after the transplantation of the cells and first dose of cyclophosphamide (50 mg/kg) was given to the same group 30 minutes after the administration of flower extract. Four more doses were given similarly on alternate days. The third group received 50mg/kg flower extract alone for 5 alternate days. The fourth group was given *Saraca asoka* bark extract (50mg/kg) and CTX similar to the second group of animals. The fifth group received bark extract alone and sixth group was the control which received the same volume of normal saline. The mean life span of the animals in all groups were noted and percentage increase in life span was calculated using the formula [(T-C)/C]x100 Where T is the mean survival time of animals of group two or group four and C is the mean survival time of animals of group-1.

Biochemical Studies

The blood for biochemical analysis was collected from caudal vein on day 3, day 6 and day 10. WBC was performed using Neubauer hemocytometre and haemoglobin by Drakkin's method. The SGPT levels and SAKP levels were determined by dinitrophenyl hydrazine method and 4-amino antipyrene methods respectively.

Statistical Analysis

Students t-test was employed to calculate statistical significance.

3. RESULTS

The combined administration of either *Saraca asoka* flower (50mg/kg) or bark (50mg/kg) extracts significantly increased the life span of mice treated with chronic lethal doses Cyclophosphamide (50mg/kg) ($P>0.05$and $P>0.01$respectively). The mean survival time

MST of mice receiving combined treatment was increased by almost 2 fold as compared to mice receiving cyclophosphamide alone (Table I).

Table I. Effect of *Saraca asoka* (Asoka) flower and bark extracts on the lifespan of sarcoma-180 tumour bearing mice treated with cyclophosphamide.

	Treatment	Mean ±SD Survival time(days)	Increase in life span %	P*
1	S-180 + cyclophosphamide (50mg/kg)	+37.82 ± 1.6	-	
2	S-180 + cyclophosphamide (50mg/kg) + Asoka flower extract (50mg/kg)	+69.22 ± 2.95	83.02	P<0.05
3	S-180 + Flower(50mg/b.wt)	+59.32 ± 2.47	56.84	
4	S-180 + cyclophosphamide (50mg/kg) + Asoka bark extract (50mg/kg)	+88.56 ± 1.91	134.16	P<0.01
5	S-180 + Asoka bark(50mg/kg)	+63.36 ± 1.30	67.53	
6	S-180 alone	+18.33 ± 1.210	-	

Tabular values represent the mean ± SD of seven animals per group for three separate experiments (N=21). Statistically significance from cyclophosphamide treated mice (P*)

ILS = [(T-C)/C] X 100%

T – Protected group

C-Control

Cyclophosphamide administration produced a gradual decline in the total WBC counts and haemoglobin levels (Table II, Table III). The extracts of flower and bark increased the WBC counts and haemoglobin levels when given along with cyclophosphamide. However the asoka bark extract, was more effective in elevating WBC counts.

Table II. Effects of Asoka (*Saraca asoka*) flower and bark extracts on the total leucocyte counts of sarcoma-180tumour bearing mice treated with or without cyclophosphamide.

	Treatment	Total leucocyte counts(cells/mm 3)			Maximum change (%) C
		Day 3	Day 6	Day 10	
1	S-180 + cyclophosphamide (50mg/kg)	9137 ± 311.21	6166±404.14	2540±314.32	-72.2
2	S-180 + cyclophosphamide (50mg/kg) + Asoka flower extract (50mg/kg)	10393 ± 460.57	8050 ± 834	6316 ± 763.70*	-39.2
3	S-180 + Asoka flower (50mg/b.wt)	10828 ± 114.20	10100 ± 200	9688 ± 832	-10.53
4	S-180 + cyclophosphamide (50mg/kg) + Asoka bark extract (50mg/kg)	10331 ± 361.48	8666 ± 808	7408.66 ± 210.01*	-28.29
5	S-180 + Asoka bark (50mg/kg)	10,594 ± 425.00	9300 ± 264.57	9066 ± 960	-14.42
6	S-180 alone	10537.66 ± 384.31	9906.66 ± 300.12	9413 ± 149.03	-10.66

Values represent the mean ± SD of seven mice per group for three experiments statistically significant from cyclophosmide treated mice. *P<0.

Table III. Effects of Asoka (*Saraca asoka*) flower and bark extracts on the haemoglobin levels of sarcoma – 180 bearing mice treated with or without cyclophosphamide.

	Treatment	Total leucocyte counts(cells/mm 3)			Maximum change (%) C
		Day 3	Day 6	Day 10	
1	S-180 + cyclophosphamide (50mg/kg)	13.90 ±0.45	9.15 ±0.919	8.36 ±0.90	-40
2	S-180 + cyclophosphamide (50mg/kg) + Asoka flower extract (50mg/kg)	13.40 ±0.45	11.46 ±0.305	11.06 ±0.513**	-17.46
3	S-180 + Asoka flower (50mg/b.wt)	13.23 ±0.25	12.5 ±1.32	11.9 ±0.26	-10.05
4	S-180 + cyclophosphamide (50mg/kg) + Asoka bark extract (50mg/kg)	12.93 ±0.23	12.76 ±0.326	11.86 ±0.68*	-8.50
5	S-180 + Asoka bark (50mg/kg)	13.06 ±0.70	12.86 ±0.68	12.56 ±0.6	-3.82
6	S-180 alone	14.46 ± 0.25	13.25 ±0.6	13.0 ±0.6	-10.9

Values represent the mean ± SD of seven mice per group for three experiments statistically significant from control tumour bearing(S-180) mice. **P<0.001 *P<0.05.

Table IV. Effects of Asoka (*Saraca asoka*) flower and bark extracts on SGPT and SAKP levels of sarcoma-180bearing mice treated with or without cyclophosphamide.

	Treatment	Serum glutamate pyruvate transaminase levels (IU/L)		Serum alkaline Phosphatase levels (KA Units)	
		Day 4	Day 10	Day 4	Day 10
1	S-180 + cyclophosphamide (50mg/kg)	10.42 ± 0.45	12.3 ± 0.25	5.9 ± 0.25	8.80 ± 0.55
2	S-180 + cyclophosphamide (50mg/kg) + Asoka flower extract (50mg/kg)	6.72 ± 0.43	7.9 ± 0.7*	5.8 ± 0.40	5.26 ± 0.115**
3	S-180 + Asoka flower (50mg/b.wt)	6.33 ± 0.41	7.26 ± 0.50	4.3 ± 0.36	4.6 ± 0.20
4	S-180 + cyclophosphamide 50mg/kg + Asoka bark extract 50mg/kg	5.13 ± 0.30	6.26 ± 0.30**	4.23 ± 0.152	5.4 ± 0.20
5	S-180 + Asoka bark (50mg/kg)	5.2 ± 0.6	5.83 ± 0.404	4.23 ± 0.152	5.2 ± 0.7
6	S-180 alone	4.40 ± 0.30	4.6 ± 0.20	4.1 ± 0.25	4.1 ± 0.2

Values are mean ± SD from three experiments. **P - < .001 *P - <0.01.

4. DISCUSSION

Asoka *(Saraca asoka*) is a common drug used to treat various diseases by the Sri Lankan and Indian systems of medicine.[12,14] We reported on the cytotoxic and the antitumour activity of Asoka against a variety of murine tumour models.[8] The chemical analysisof the active compound from the bark extract of Asoka showed that (-)- epicatechin was responsible for the anti tumour/ anti carcinogenic activity.[12,13] The aim of our investigation was to find out whether the toxic manifestations of cyclophosphamide could be reduced by the co-administration of extracts of *Saraca asoka* flower and bark. The protected mice maintained near normal WBC and haemoglobin levels as compared to cyclophosphamide treated groups indicating protective effect of these extracts at the hematopoietic level. A similar result was reported.[4] using 2- mercapto propionyl glycine (2-MPG). The decreased serum glutamate pyruvate and serum alkaline phosphatase levels in the protected groups further indicate protection against hepatic toxicity.

5. CONCLUSION

The anticancer activity of cyclophosphamide was not affected by the co- administration of these natural products as evidenced by the increase in the life span of tumour bearing mice

that received combined treatment. However, better protective effects were observed in animals treated with bark extract. The exact mechanism by which (-) - epicatechin from bark extract of Asoka induces chemoprotective effects in the above studies remains to be further investigated.

ACKNOWLEDGEMENT

The author wish to thank the Amala Cancer Hospital for the facilities. The Indian Council Medical Research (New Delhi) is acknowledged for the financial support through their project.

6. REFERENCES

1. Sauhami, R. L., Harper, P.G., Linch, D., Traske, F. A. H. and Richards, J. D. High dose of Cyclophosphamide with autologous marrow transplantation as initial treatment, small cell carcinoma of the bronches cancer. Chemother, Pharmacol, 1982; 8(31).
2. Sladek, N. E. and Landkamer, G. J. Restoration of sensitivity to oxanzaphosphorines by inhibitors of aldehyde dehydrogenase activity on cultured ocazaphorine resistant L1210 and cross linking against resistant, Cancer Res, 1985; 4(5): 1549.
3. Wrabtz, E, Peter, G. and Rohorst, H. J. Does Acrolein Contribute t the cytoxicity of Cyclophosphamide. J Cancer Res. Clin. Oncol, 1980; 98: 119.
4. Philip, L. T., Yuhan, J. M. and Wasserman, T. H. Role of 2- mercaptopionyl glycine (MPG) against toxicity of Cyclophosphamide in normal and tumour bearing mice. Ind. J Exp. Bio, 1986; 24: 767.
5. Nair, S. C. Salomi, M. J. and Panikkar, K. R. Chemoprotective effects of some Natural Products. Proc. Ntl. Serno Cancer, 1990; 28.
6. Unnikrishnan, M.C., Soudamini, K. K. and Ramadasan K. Chemoprotection of Garlic Extract towards CTX toxicity in mice, Nutr. Cancer, 1989; 13: 201.
7. Pradhan, P., Joseph, L., Gupta, V., Chulet, R., Arya, H., Verma, R. and Bajpai, A. Saraca asoka (Asoka): A Review. Journal of Chemical and Pharmaceutical Research, 2009; 1(1): 62.
8. Varghese, C.D. Nair, S.C. and Panikkar, K.R. Potential anticancer activity of Saraca asoka extract towards transplantable tumors in mice. Ind. J. Pharm.Sci, 1992; 54: 37.
9. Govindarajan, R., Singh, D. P. and Rawat, A. K. S. Validated RP-LC method for standardization of Ashokarishta: A polyherbal formulation. Chromatographia, 2008; 68: 873.

10. Middelkoop, T.B. and Labadie, R. P. Pro anthocyanidins in the bark of Saraca asoka. Z. Naturforsch, 1985; 40b: 855.
11. Nair, S.C. and Panikkar, K.R., Antitumour principles from Ixora javanica. Cancer Lett., 1990; 49: 121.
12. 12 Middelkoop, T. B. and Labadiei R. P. Evaluation of Asoka Aristha, An Indigenous medicine in Sri Lanka, Journal of Ethno Pharmacology, 1983; 8: 313.
13. Varghese, C. D., Nair, S. C., Panikkar, B. and Panikkar, K. R. Effect of asoka on the intracellular glutathione levels and skin tumour promotion in mice. Cancer Letters, 1993; 69: 45.
14. Tannock, I. F. Experimental chemotherapy In: The Basic Science of Oncology, 1987; 308-325.
15. Colvin, N. The alkylating agents. In: Pharmacological principles of cancer treatment, 1982; 294-298. Editors: Chabner, B. Philadelphia, W.B. Sounders Co.
16. Nadkarni, K. M. Indian Meteria Medica, 3rd edn., Popular Book Depot, Bombay, 1954; 1105.