

Modulatory effects of *Ixora coccinea* flower on Cyclophosphamide toxicity in tumour bearing mice.

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ABSTRACT : The active fraction (AF) from *Ixora coccinea* flowers prevented the decrease in haemoglobin levels and leucocyte counts of Dalton's lymphoma tumour bearing mice, treated with cyclophosphamide (CYP). It also significantly increased the life span of tumour bearing mice, treated with cyclophosphamide. Serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (SAKP) levels of tumour bearing mice treated with CYP were decreased significantly by combination therapy with *I.coccinea* AF. indicating protection against hepatic toxicity.

INTRODUCTION

Cyclophosphamide (CYP), an alkylating agent is a broad spectrum anti - cancer drug, which has been shown to be inactive in vitro, but was found to be activated in the liver by the microsomal enzyme system in the presence of oxygen and reduced NADPH to cytotoxic metabolites^{1,2}. The high therapeutic index of CYP was shown to be due to the metabolite, 4-hydroxycyclophosphamide and a phosphoramidate derivative³. It was shown that free radicals are formed due to the activation of CYP and produce tissue injury⁴. Toxic syndromes of CYP include the suppression of white blood cells, nausea, vomiting, gonadal atrophy and renal and bladder injury. It has been reported that sulphhydryl agents such as MPG (2-mercaptopyrionylglycine) and WR 2721 (aminoethylphosphorothioic acid) reduce the toxicity of CYP by preventing free radical accumulation and promoting repair⁵. Considerable interest has been focused on the isolation of phytochemicals that might be administered along with CYP to reduce its toxicity. The flowers of *Ixora coccinea* L. (Rubiaceae) are extensively used in

Ayurvedic medicine⁶. The cytotoxic and antitumour properties of the flowers have been studied⁷. We have already reported the chemoprotective effect of *I. Coccinea* flowers on CYP - induced toxicity in normal mice⁸. In this paper, the protective effects of the active fraction of *I.coccinea* flowers against CYP induced toxicity in Dalton's Lymphoma Ascites (DLA) tumour bearing mice is reported.

MATERIALS AND METHODS

Chemicals

CYP (Endoxan Asta) was purchased from German Remedies Limited, Thane, Mumbai. It was diluted to the required concentrations with distilled water. All other chemicals and reagents were of analytical grade, from Sisco Research Laboratories, Mumbai.

Plant material and extraction procedure

I. coccinea flowers were obtained from the herbal garden of the Institute, and authenticated by Dr.N.Mohanam, plant

taxonomist. A voucher specimen had been deposited at the herbarium of the Institute (Accession No.8735 of 21/1/1992)

The preparation of the active fraction (AF)

The AF of *I. coccinea* flowers was prepared as described elsewhere⁷. Briefly, the shade - dried flowers were powdered and 100g of the powder was extracted with 500ml n-hexane in a Soxhlet for 48h. This was evaporated under reduced pressure to obtain a yellow residue.(4.5 g). The residue was loaded on a Silica gel column and eluted with hexane - 5% ethyl acetate mixture. This fraction was concentrated *in vacuo* to obtain a white powder (450 mg).This is the AF. It was reconstituted in 6 mM phosphate buffer saline (PBS, pH 7.4), containing 0.04% Tween - 80 and used for the experiments.

Animals

Swiss albino mice, males, 8-10 weeks old, weighing 25-30g were obtained from the animal house of the Institute. They were maintained under standard laboratory conditions.

Tumour cells

DLA tumour cells propagated i.p., in mice were obtained from Amala Cancer Research Centre, Thrissur, Kerala.

CYP treatment of tumour - bearing mice

Four groups of mice (7/group) were used for the study. One million tumour cells were transplanted i.p., into all the 4 groups.

24h after tumour transplantation, CYP (50 mg/kg) was injected i.p., for 5 alternate days to the animals of the first group. The second group was given i.p., 100 mg/kg of *I.coccinea* AF, and 30 min before CYP

treatment. The third group was given *I.coccinea* AF (100mg/kg) only and the fourth group was the control, which received PBS containing 0.04% Tween-80. The treatment was continued for 4 alternate days. The mortality due to tumour burden and CYP toxicity was recorded daily. The percentage increase in life span (ILS %) of the animals was calculated. The experiment was repeated twice.

Haematological and biochemical studies

Peripheral blood for the various assays was collected from the caudal vein at different levels. Total leucocyte counts were made using a haemocytometer. Haemoglobin content was measured using Sahli's haemoglobinometer. The serum glutamate pyruvate transaminase (SGPT) and serum alkaline phosphatase (SAKP) levels were determined by standard methods⁹⁻¹⁰.

Acute toxicity study

Mice were fasted overnight but was given boiled water *ad libitum*. Doses of 100, 200 and 400mg /kg of the AF were administered i.p., to the animals. They were observed for toxic symptoms, during a 24h period.

Statistical analysis

Differences between control and treated animals were evaluated for significance by Student's 't' test.¹¹

RESULTS AND DISCUSSION

Table 1 shows the increase in life span of DLA tumour bearing animals treated with CYP and *I.coccinea* AF. There was a 48% increase in life span of the combination treated groups, compared to the CYP treated tumour group (Table 1).

CYP treatment gradually lowered the total leucocyte count and haemoglobin levels in tumour bearing mice. But these levels were improved significantly in tumour bearing animals given *I.coccinea* along with CYP (Figs 1,2)

The SGPT and SAKP levels were significantly increased in the CYP treated tumour group. The combination therapy with CYP and *I.coccinea* AF reduced SGPT and SAKP levels, compared to CYP treated tumour controls (Table 2)

In the acute toxicity study, no mortality occurred within 24h with any of the doses of AF tested. The treated animals did not show any changes in general behaviour during the study.

In the present study, *I.coccinea* AF was found to reduce CYP induced toxicity in tumour bearing mice. The combination treatment of CYP and *I.coccinea* AF on tumour bearing mice resulted in an increase in life span, which indicated the chemoprotective effect of the AF. Lowered levels of SGPT and SAKP in the combination treated group revealed protection by AF against liver necrosis. SGPT and SAKP are sensitive tests to detect hepatic disease. Elevated levels of serum enzymes are indicator of cellular leakage and loss of functional integrity of cell membranes of the liver¹². CYP is activated in the liver to cytotoxic metabolites². Free radicals are formed during the activation of CYP and cause liver tissue injury⁴, leading to increased SAKP and SGPT levels as

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observed in the present study. Therefore the present study suggests that *I.coccinea* AF reduces the toxicity of CYP by preventing free radical accumulation and promoting repair. Previous studies^{13,14} had shown that *I.coccinea* AF contained the triterpenoid ursolic acid, which has very potent hepatoprotective effects, comparable to that of silymarin. This is due to its membrane stabilising and anti - oxidant effects¹⁵. Ursolic acid is known to protect from liver injury in mice¹⁶.

I.coccinea AF is non - toxic, from the results of our study. This is not surprising as it is an ingredient of several Ayurvedic formulations.

Our results also showed that *I.coccinea* protected the haemopoetic system like the blood cells (RBC, WBC) and the bone marrow, which is the primary site of blood cell formation¹⁷.

I.coccinea AF is reported to possess anti tumour properties⁷. Therefore it helped to reduce the tumour burden by itself and also helped to reduce the toxicity of the anti - cancer drug, CYP, when used in combination with it.

The present study thus warrants use of *I.coccinea* AF in combination chemotherapy with CYP in the treatment of malignancy.

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TABLE - 1

Effect of *I. coccinea* flower AF on the lifespan of DLA tumour bearing mice treated with cyclophosphamide (CYP)

Treatment	Mean survival time (Days)	% increase in life span (% ILS)
DLA (Control)	25.57 ± 3.18	-
DLA + CYP (50 mg/kg)	40.42 ± 2.54	-
DLA + CYP (50mg/kg)+ <i>I. coccinea</i> (100 mg / kg)	59.00 ± 3.16	47.85 *

* P ≤ 0.05 vs CYP control, n = 21

TABLE - 2

Effect of *I.coccinea* AF on the serum enzymes of CYP treated DLA tumour bearing mice.

Treatment	SGPT (IU/L)		SAKP (KA units)	
	Day 3	Day 7	Day 3	Day 7
DLA (control)	6.8 ± 0.02	5.9 ± 0.62	4.1 ± 0.63	4.8 ± 0.73
DLA + CYP (50 mg / kg)	128.8 ± 1.23	16.9 ± 2.09	7.3 ± 0.97	8.3 ± 1.11
DLA + CYP (50mg / kg) + <i>I.coccinea</i> (100mg / kg)	8.9 ± 1.23*	12.8 ± 2.09*	5.2 ± 0.73*	6.1 ± 1.24*
DLA + <i>I.coccinea</i> (100 mg/kg)	6.6 ± 0.61	6.9 ± 0.64	4.9 ± 0.75	5.9 ± 0.57

* P ≤ 0.05 vs CYP control, n = 21

Legends to figures

Fig. 1 - Total leucocyte count of DLA tumour bearing mice, treated with CYP (50 mg / kg), with or without *I.coccinea*. Values are mean + SD, n=21 **P<0.01,vs.DLA + CYP

Fig. 2 - Haemoglobin level of DLA tumour bearing mice, treated with CYP (50mg/kg), with or without *I.coccinea*. Values are mean_± SD, n=21. **P<0.01, vs.DLA + CYP

