

**PROTECTION OF CYCLOPHOSPHAMIDE INDUCED
MYELOSUPPRESSION BY ALCOHOLIC EXTRACT OF *PIMENTA DIOICA*
LEAVES IN MICE**

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Summary

The leaves of *Pimenta dioica* (L.) Merril (Fam: Myrtaceae) were used as medicine for pain, arthritis, fever and stress in the folklore medicine at Kerala and Mangalore. The berries are commonly known as Allspice in culinary natives of Kerala and Mangalore. The high total phenolic content in the berries and leaves were reported for antioxidant activity. In the present study we tried the alcoholic extract of leaves for protection in cyclophosphamide induced myelosuppression in Swiss mice. The oral doses (250, 500, 750 mg/kg/day) showed statistically significant protection of myelosuppression.

Key words: Allspice, *Pimenta dioica*, Myelosuppression, Antioxidant

Introduction

Berries of *Pimenta dioica* (L.) Merril (Fam: Myrtaceae) is commonly known as Allspice in culinary. It takes its name from the aroma of dried berries, which smells like the combination of spices, especially cinnamon, cloves, ginger and nutmeg. Allspice owes its characteristic odour to the presence of essential oil in the pericarp of the seeds. The plant Allspice is mentioned in Wealth of India (1). The dried leaves contain 0.7 to 2.9 % of oil which is called pimento oil. Like berry oil it contains eugenol as its main constituent but has an inferior odour and flavour. Phytochemistry and pharmacology of berries were reported in many literatures (2-7) but few on leaves. The natives of Kerala and Mangalore use Allspice leaves as medicine for pain, arthritis, fever and stress. In all these pathological conditions oxidative stress is one of the causes, which made us to explore the leaves extract for in vitro antioxidant

activity. Antioxidant and hepatoprotective activity in CCl₄ induced liver toxicity of Allspice leaves reported earlier (8). We found the changes in the hematological parameters during our studies which made us to study for protection in myelosuppression.

Antioxidants from natural or synthetic origin act by preventing free radical formation, scavenging free radicals, facilitating the repair caused by free radicals or by favoring the antioxidant defense in the body (9). Plant sources like turmeric, ginger, garlic, onion, clove, grape seeds, apple, ginkgobiloba and fruit peels like lemon, orange have got strong antioxidants (10). Antioxidants are beneficial in preventing disease complexes such as cardiovascular, diabetes, cancer, rheumatoid arthritis, inflammatory bowel disease, pancreatitis, haematological and neurodegenerative diseases (11-12). Vitamins (A, C, E), glutathione, lycopene, α -lipoic acid, L-Cystine, L-methionine and micronutrients such as zinc, selenium and chromium are beneficial in oxidative stress induced diseases for prevention and alleviation (13).

Materials and Methods

Chemicals

Chemicals and reagents used for the experiments were all AR grade, purchased from Sigma, HiMedia, NICE and Loba chemicals. In animal studies for oral administration the extract was made into suspension with 0.5% carboxy-methyl cellulose (CMC).

Plant material

Pimenta dioica (L.) Merril (Fam: Myrtaceae) was identified and authenticated by the taxonomist Dr. Pradeep, Dept of Botany, NSS College, Vazhoor, Kerala. Fresh leaves collected were cleaned and washed with water. Weighed initially and dried under shade at room temperature for constant weight. It was found to loose 70% weight and the leaves became brittle in nature. Dried leaves were then powdered using mixer grinder and stored at 4°C in refrigerator before it was used for the alcoholic extraction.

Alcoholic Extraction

Powdered leaves of *Pimenta dioica* was extracted with 70%V/V ethanol by continuous extraction in soxhlet apparatus. Each batch was extracted for 40 cycles, and then concentrated in a flash evaporator under reduced pressure and temperature. The semisolid product obtained was dried in vacuum desiccators, stored at 4°C. Further, phytoconstituent were tested by standard methods (14).

Animals

In-bred Swiss albino mice maintained under controlled conditions of temperature ($23 \pm 2^\circ\text{C}$) and humidity ($50 \pm 5\%$) and a 12-hour light–dark cycle, weighing between 20 to 30 g were used for the experiment. They were housed in sanitised polypropylene cages containing sterile paddy husk as bedding. They had free access to standard

mouse food and water *ad libitum*. The studies were conducted with the prior approval of Institutional Animal Ethics Committee as accordance with guidelines of Committee for the Purpose of Control and Supervision of Experiments on Animals (CPCSEA), Government of India.

Cyclophosphamide induced myelosuppression

Swiss mice of either sex (20 to 30 gm) were used selected and distributed into 5 groups (n=6). Positive and negative control group animals received 0.5 % CMC 0.3 ml/kg, p.o. daily for 14 days. Animals in the treatment groups were given the test extracts 250, 500 and 750 mg/kg, p.o., in 0.5% W/V CMC daily for 14 days. On days 11, 12, 13 all the animals except in the negative control group were injected with cyclophosphamide 30 mg/kg, i.p., one hour after the administration of extracts. Haemoglobin concentration and WBC count was done on 1st, 10th and 14th day for each group animal. Blood was collected from the tail vein puncture. Data analysed by ANOVA and Dunnet's "t" test using Graphpad Prism.

Result and discussion

Table.1. Effect of Alcoholic extract of Allspice leaves on WBC count in Cyclophosphamide induced Mice

Treatment Group n=6	Total WBC Count (mm ³)		
	Day 1	Day 10	Day 14
Group-I	6002 ± 512	6073 ± 570	6068 ± 438
Group-II	6050 ± 630	6005 ± 350	3504 ± 331 ^b
Group-III	5900 ± 480	6902 ± 320 ^{b,c}	6604 ± 442 ^{b,c}
Group-IV	5859 ± 590	7107 ± 350 ^{b,c}	6902 ± 421 ^{b,c}
Group-V	6092 ± 460	7350 ± 260 ^{b,c}	7104 ± 350 ^{b,c}

Table.2. Effect of Alcoholic extract of Allspice leaves on Haemoglobin concentration in Cyclophosphamide induced Mice

Treatment group n=6	Haemoglobin content (gm/dl)		
	Day 1	Day 10	Day 14
Group-I	13.020 ± 0.320	12.01 ± 0.420	13.80 ± 0.440
Group-II	12.090 ± 0.540	13.10 ± 0.440	8.03 ± 0.430 ^a
Group-III	14.080 ± 0.40	14.30 ± 0.63 ^{b,c}	14.10 ± 0.53 ^{b,c}
Group-IV	13.30 ± 0.460	14.50 ± 0.470 ^{b,c}	14.40 ± 0.670 ^{b,c}
Group-V	14.10 ± 0.320	14.60 ± 0.540 ^{b,c}	14.52 ± 0.510 ^{b,c}

Values are Mean ± SEM; ^a = p<0.05 Vs control (group I); ^b = p< 0.05 Vs cyclophosphamide induced control (group II); Treatments: Group-I (negative control): 0.5% CMC for 14 days; Group-II (positive control): 0.5% CMC for 14 days + Cyclophosphamide on 11, 12, 13 day; Group III to V: Extract at doses 250, 500, 750 mg/kg for 14 days + Cyclophosphamide on 11, 12, 13 day

Cyclophosphamide is an anticancer drug the major side effect of this drug is immune and hematological suppression. The method was followed as discussed earlier in the literature (15).

We found that the WBC count decreased drastically after treatment with the cyclophosphamide i.e. 6050 ± 630 to 3504 ± 331 cells/mm³. On the other hand decrease in the haemoglobin concentration (i.e. 13.100 ± 0.440 to 8.030 ± 0.430) was the indication of immune suppression produced by cyclophosphamide at concentration of 30mg/kg i.p. Allspice treated animals have shown increase in the WBC count and haemoglobin concentration after 10 days of treatment at the dose levels of 250, 500 and 750mg/kg/day p.o.

This increase in the WBC and haemoglobin concentration may be because of the immune stimulant properties of the Allspice leaves. When the same animals were induced the cyclophosphamide toxicity on the day 11, 12 and 13, there was no decrease in the WBC count and haemoglobin concentration. Thus complete protection was observed.

Conclusions

The Allspice leaves extract showed good protection in cyclophosphamide induced myelosuppression could be because of the total phenolic compounds. The eugenol is found to be the major component in the leaves may be responsible for the activity. The total radical scavenging property could also be reasonable for the myelo-stimulant property. The study has to be continued to investigate the mechanistic approach of the leaves by better *in vitro* and *in vivo* models.

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