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ANTIMUTAGENIC EFFECTS OF SOME ANTICANCER AGENTS*

C. Y. Lim-Sylianco and F. Blanco

ABSTRACT

Amygdalin, cyclophosphamide, and decoction of leaves of *Catharanthus roseus* (Linn.) Don. were studied as regards to their antimutagenic effects, using the micronucleus technique. The test mutagens that were employed were dipyrone, metronidazole, mitomycin C and safrole.

Amygdalin, cyclophosphamide, and decoction from leaves of *Catharanthus roseus* (Linn.) Don. reduced the number of micronucleated polychromatic erythrocytes caused by the test mutagens. Highest antimutagenic effect was observed with decoction from leaves of *Catharanthus roseus* (Linn.) Don. Weakest effect was given by cyclophosphamide. An intermediate effect was shown by amygdalin.

INTRODUCTION

There is global concern about environmental agents that interact with DNA of living cells. When DNA of somatic cells is altered and the defect is not repaired, neoplasm may result after a latent period. Thus, mutagenic and carcinogenic potentials of many environmental agents are under close scrutiny.

Several environmental mutagens are implicated in the genesis of cancer. For this reason, it is of practical interest to find out if anticancer agents exhibit antimutagenic effects.

In this connection, antimutagenic effects of three anticancer agents are reported. These are amygdalin, cyclophosphamide, and decoction from leaves of *Catharanthus roseus* (Linn.) Don. The test mutagens are dipyrone, metro nidazole, mitomycin C, and safrole.

Dipyrone has been shown to be mutagenic before and after metabolic activation (1). Metronidazole, an active principle of a well-known antichromonal agent, has been shown to increase mutation frequency of bacteria several times above the spontaneous mutation rate (2). It has been reported to induce lung cancer in mice (3).

Amygdalin (4) occurs in bitter almonds, peaches, and apricots. It has been reported to be used in cancer chemotherapy since 1845 but there is absence of data substantiating its anticancer activity (5).

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Cyclophosphamide has been observed to induce chromosome breakage in vivo (6) and also sister chromatid exchange in rabbit lymphocytes (7).

Catharanthus roseus (Linn.) Don. has been reported to contain vincristine (8). Although considered as an anticancer agent, vincristine did not increase chromosome damage in rats (9).

MATERIALS AND METHODS

Dipyrone, metronidazole, and mitomycin C were obtained from a local drugstore. Safrole was an Aldrich product. Cyclophosphamide was a gift from Philippine FDA. Amygdalin was purchased from CALBIOCHEM. The mice used were of the Swiss Webster strain.

The micronucleus technique of W. Schmid (10) was used in these studies. Briefly, the highest tolerated dose of mutagens (in dimethyl sulfoxide) was given intraperitoneally to experimental mice while amygdalin, cyclophosphamide and decoction of leaves of *Catharanthus roseus* (Linn.) Don. were given orally. Administration was done twice, 30 hours and 6 hours prior to the preparation of the bone marrow. Bone marrow of the femur was flushed into a test tube containing fetal calf serum and centrifuged. The air-dried smear was stained and examined for micronuclei in erythrocytes.

RESULTS AND DISCUSSION

Dipyrone, metronidazole, mitomycin C, and safrole, in maximum tolerated dosages, caused the formation of micronuclei in erythrocytes of experimental mice (Table 1). This indicates that these substances affected the DNA of the chromatin material of dividing red blood cells. This effect led to the displacement of some chromatin fragments which formed micronuclei in the cytoplasm of these cells. Thus, not only are these substances mutagenic but also clastogenic.

Mitomycin C is a potential alkylating agent of DNA (11). Safrole has also been shown to be metabolized to an alkylating agent (12). It is possible that their mutagenic and clastogenic effects are consequences of their alkylating ability.

Amygdalin, cyclophosphamide, and decoction from leaves of *Catharanthus roseus* (Linn.) Don. reduced micronuclei formation caused by dipyrone, metronidazole, mitomycin C, and safrole. These, therefore, have antimutagenic and anticlastogenic effects. It is possible that these antimutagens reduced the alkylating ability of mitomycin C and safrole.

The highest reduction in micronuclei formation was observed with decoction of leaves of *Catharanthus roseus* (Linn.) Don. Of the three systems tested, this is the best antimutagen. Cyclophosphamide gave the poorest antimutagenic response.

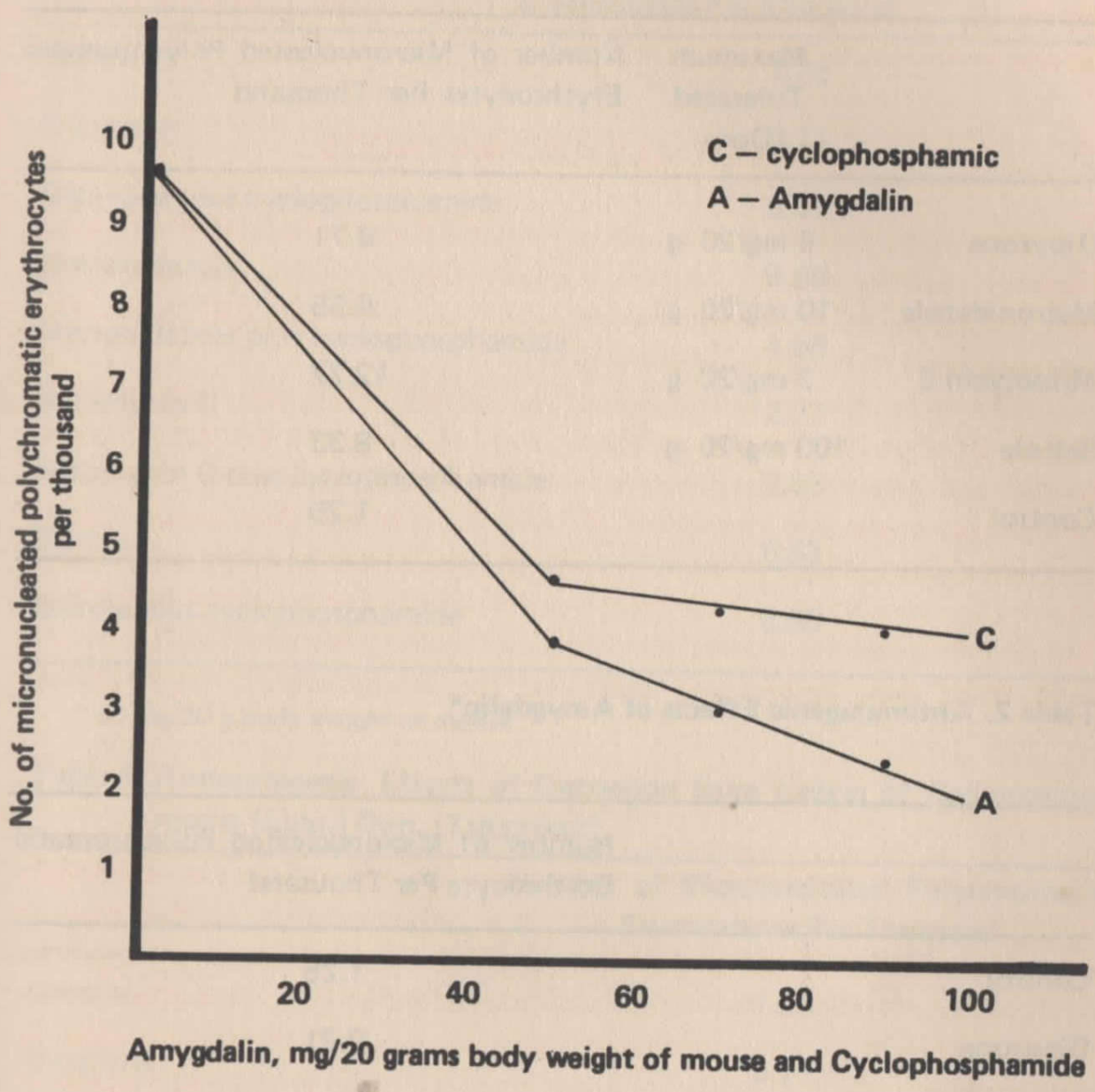

Figure 1. Effect of Dosage on the Antimutagenic Effects of Amygdalin and Cyclophosphamide on Metronidazole

The antimutagenic effect of amygdalin was dose-dependent (Fig. 1). Infusion from Tsitsirika leaves showed weaker antimutagenic effect than the decoction (Table 5).

Table 1. Mutagenic and Clastogenic Effects of Dipyrone, Metronidazole, Mitomycin C, and Safrole on Bone Marrow of Experimental Mice.

	Maximum Tolerated Dose	Number of Micronucleated Polychromatic Erythrocytes Per Thousand
Dipyrone	5 mg/20 g	9.71
Metronidazole	10 mg/20 g	9.55
Mitomycin C	3 mg/20 g	12.77
Safrole	100 mg/20 g	8.33
Control		1.25

Table 2. Antimutagenic Effects of Amygdalin*

	Number of Micronucleated Polychromatic Erythrocyte Per Thousand
Control	1.25
Dipyrone	9.71
Dipyrone plus amygdalin	5.13
Metronidazole	9.55
Metronidazole plus amygdalin	3.80
Mitomycin C	12.77
Mitomycin C plus amygdalin	7.06
Safrole	8.33
Safrole plus amygdalin	5.66

* 50 mg/20 g body weight of mouse

Table 3. Antimutagenic Effects of Cyclophosphamide*

	Number of Micronucleated Polychromatic Erythrocytes Per Thousand
Control	1.25
Dipyrone	9.71
Dipyrone plus cyclophosphamide	6.08
Metronidazole	9.55
Metronidazole plus cyclophosphamide	4.66
Mitomycin C	12.77
Mitomycin C plus cyclophosphamide	7.33
Safrole	8.33
Safrole plus cyclophosphamide	6.55

* 50 mg/20 g body weight of mouse.

Table 4. Antimutagenic Effects of Decoction from Leaves of *Catharanthus roseus* (Linn.) Don. (Tsitsirika)*

	Number of Micronucleated Polychromatic Erythrocytes Per Thousand
Control	1.25
Dipyrone	9.71
Dipyrone plus Tsitsirika	3.22
Metronidazole	9.55
Metronidazole plus Tsitsirika	2.33
Mitomycin C	12.77
Mitomycin C plus Tsitsirika	3.55
Safrole	8.33
Safrole plus Tsitsirika	2.22

* 5% decoction

Table 5. A Comparison of Antimutagenic Effects of Decoction and Infusion from Leaves of *Catharanthus roseus* (Linn.) Don. (Tsitsirika).

	Number of polychromatic Micronucleated Erythrocytes Per Thousand
Metronidazole	9.55
Metronidazole plus Tsitsirika decoction	2.33
Metronidazole plus Tsitsirika infusion	6.88

CONCLUSION

Three anticancer agents, amygdalin, cyclophosphamide, and decoction from leaves of *Catharanthus roseus* (Linn.) Don. exhibited antimutagenic effects against dipyrone, metronidazole, mitomycin C and safrole.

Of the three, decoction from the leaves of *Catharanthus roseus* (Linn.) Don. exhibited the best antimutagenic effect. Weakest antimutagenic effect was given by cyclophosphamide. Amygdalin showed intermediate antimutagenic effects.

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