# Antigenotoxic Effects on Bone Marrow Cells of Refined Coconut Oil Administered Simultaneously With Azaserine, Dimethylhydrazine, Dimethylnitrosamine, Benzo(a)pyrene, Methylmethanesulfonate and Tetracycline*

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ABSTRACT

*Methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a)pyrene, azaserine and tetracycline induced the formation of micronucleated polychromatic erythrocytes in bone marrow cells of experimental mice, an indication that these chemicals exhibited chromosome-breaking effects and are therefore, genotoxic.*

*Simultaneous administration of refined coconut oil reduced micronuclei formation indicating that coconut oil has antigenotoxic activity against methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a)pyrene, azaserine and tetracycline. The antigenotoxic activity of refined coconut oil was far superior to that of soybean oil (low and high peroxide value)*

* Supported by the Philippine Coconut Research and Development Foundation

## INTRODUCTION

It has been shown that (1), methylmethanesulfonate, (2), di- methylnitrosamine, (3), dimethylhydrazine, (4), azaserine and (5), benzo(a)pyrene are chemical carcinogens that alter the structure of DNA, the genetic substance of the living cell. Tetracycline is a well known teratogen (6), Azaserine can induce pancreatic carci- noma while 1,2-dimethylhydrazine is a colon carcinogen. Benzo- (a)pyrene can induce skin and liver cancer. Methylmethanesulfonate and dimethylnitrosamine are carcinogens which alkylate DNA (7).

Studies published several years ago suggested the inhibitory effects of dietary coconut oil on the development of colon tumors (8), liver tumors (9), pancreatic carcinoma (4), mammary tumors (10) and skin cancer (5). However, these findings were ignored by those who have tried to malign coconut oil. These were long-term studies that were done for long periods of time.

This report deals with short-term approaches in studying the effect of coconut oil on the genotoxicity of methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a) pyrene, azaserine and tetracycline.

## MATERIALS AND METHODS

Methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, azaserine, benzo(a)pyrene and tetracycline were purchased from Sigma Chemical Company, St. Louis, Missouri, U.S.A.

Fetal calf serum was purchased from Grand Island Biological Supply, Grand Island, New York, U.S.A.

Swiss Webster mice were supplied by the College of Veterinary Medicine, University of the Philippines.

The micronucleus test (11) was used in studying the genotoxicity of methylmethanesulfonate, dimethylnitrosamine, dimethylhydrazine, benzo(a)pyrene, azaserine and tetracycline. The same method was used in determining the antigenotoxicity effects of refined coconut oil.

A completely randomized design was used in studying genotoxicity and antigenotoxicity. Ten mice were used for each

experimental group. Two administrations for each test system were done using an oral gavage, 24 hours apart. Six hours after the second administration, the experimental mouse was killed by cervical dislocation. The femur was removed and bone marrow cells were flushed out using fetal calf serum. The cell suspension was centrifuged and the supernatant discarded.

The cells were mounted on slides, stained and micronucleated polychromatic erythrocytes were counted under the microscope.

For antigenotoxic studies, refined coconut oil (0.5 ml/20 gram body weight) was administered simultaneously with the test mutacarcinogen. Refined coconut oil as well as soybean oil (low and high peroxide) were used in the antigenotoxicity trials.

Peroxide values of the test oils were analyzed using the method described by Sully (**12**). Soybean oil with high peroxide value was prepared using the method of Ault (**13**).

## RESULTS AND DISCUSSION

Significance of data indicated in Tables 1, 2 and 3 has been statistically validated using ANOVA and Duncan's multiple range test.

Table 1 shows that the clastogenic or chromosome-breaking potential of soybean oil, both low and high peroxide, is greater than that of coconut oil. The clastogenic potential of coconut oil is lower than the blank. These results suggest that coconut oil does not possess chromosome-breaking effects. This is indicated by the very small number of micronucleated polychromatic erythrocytes produced when coconut oil was given by oral gavage. The formation of micronucleated polychromatic erythrocytes is lower than that produced spontaneously.

Soybean oil, with low peroxide, has chromosome-breaking effects. Its clastogenic potential is enhanced when the peroxide content is increased. This is a consequence of the high content of linoleic acid in soybean oil. Being polyunsaturated, it easily forms peroxides and free radicals when incubated in the presence of oxygen. Peroxides and free radicals are very reactive with DNA (deoxyribonucleic acid) (**7**).

Table 2 indicates that the carcinogens (e.g. azaserine, benzo- (a)pyrene, dimethylhydrazine, dimethylnitrosamine,

methylmethanesulfonate and the teratogen, tetracycline), induced the formation of micronucleated polychromatic erythrocytes, suggesting that these test systems fragmented the chromatin material of the bone marrow cells. These test substances are therefore genotoxic to bone marrow cells.

The genotoxicity to bone marrow cells of azaserine, benzo(a)pyrene, dimethylhydrazine, dimethlynitrosamine, methlymethanesulfonate and tetracycline was inhibited by coconut oil as shown in Table 3. The inhibitory effects of coconut oil is far superior to that of soybean oil. When the peroxide value of soybean oil is increased, its inhibitory effects is reduced. These results clearly suggest that the antigenotoxic effect of coconut oil exceeds that of soybean oil. This activity of coconut oil is indicated by the highly significant reduction in the formation of micronucleated polychromatic erythrocytes in bone marrow cells.

Azaserine, benzo(a)pyrene and dimethylnitrosamine are well-known alkylating agents of DNA, the genetic substance of the living cell (**17**). This alkylating activity can induce the fragmentation of DNA of the chromosomes inducing the formation of micronucleated polychromatic erythrocytes. It is possible that coconut oil reduced the alkylating activity of these carcinogens, by a mechanism that has yet to be elucidated.

It is also possible that coconut oil enhanced the repair of DNA that was altered structurally by the test mutacarcinogens.

## CONCLUSION

Coconut oil, a saturated oil, exhibited antigenotoxic activity against five carcinogens and a teratogen. Its antigenotoxic activity is far superior to that of soybean oil, a polyunsaturated oil.

## ACKNOWLEDGMENT

Financial support from the Philippine Coconut Research and Development Foundation is gratefully acknowledged.

**Table 1. A comparison of clastogenic potential of refined coconut oil and soybean oil**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. |
|---|---|
| Coconut Oil, refined * | 1.53 ± 0.90 |
| Soybean Oil, low peroxide ** | 3.77 ± 1.71 |
| Soybean Oil, high peroxide *** | 4.52 ± 0.53 |
| Control, no oil | 1.93 ± 0.72 |

* Coconut oil = 0 peroxide value

** Soybean oil, low peroxide, 13.97 ± 3.25 meq/kg

*** Soybean oil, high peroxide, 30.60 ± 1.31 meq/kg

**Table 2. Clastogenic or chromosome-breaking effects of azaserine, benzo(a)pyrene, dimethylhydrazine, dimethylnitrosamine, methlymethanesulfonate and tetracycline**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. |
|---|---|
| Azaserine (9.4 mg/kg) | 7.63 ± 1.79 |
| Benzo(a) pyrene (47 mg/kg) | 7.30 ± 1.08 |
| Dimethylhydrazine (18 mg'kg) | 8.22 ± 1.96 |
| Dimethlynitrosamine (10 mg/kg) | 8.52 ± 1.56 |
| Methlymethanesulfonate (10 mg/kg) | 6.00 ± 1.30 |
| Tetracycline (55 mg/kg) | 8.93 ± 3.21 |
| Control (distilled water) | 1.93 ± 0.72 |

Table 3. **Effect of refined coconut oil and soybean oil on genotoxicity of azaserine, benzo(a)pyrene, dimethylhydrazine, dimethylnitrosamine, methlymethanesulfonate and tetracycline**

| | No. of micronucleated polychromatic erythrocytes per thousand ± S.D. | | |
|---|---|---|---|
| Azaserine (AZ) alone | 7.63 | ± | 1.79 |
| AZ plus coconut oil | 2.03 | ± | 0.85 |
| AZ plus soybean oil (LP) * | 3.43 | ± | 0.83 |
| AZ plus soybean oil (HP) ** | 5.59 | ± | 1.30 |
| Benzo(a)pyrene (BP) alone | 7.30 | ± | 1.08 |
| BP plus coconut oil | 3.00 | ± | 1.47 |
| BP plus soybean oil (LP) | 4.57 | ± | 1.87 |
| BP plus soybean oil (HP) | 5.59 | ± | 1.30 |
| Dimethylhydrazine (DH) alone | 8.22 | ± | 1.56 |
| DH plus coconut oil | 2.80 | ± | 1.07 |
| DH plus soybean oil (LP) | 4.63 | ± | 1.10 |
| DH plus soybean oil (HP) | 6.63 | ± | 1.46 |
| Dimethylnnitrosamine (DMN) alone | 8.52 | ± | 1.56 |
| DMN plus coconut oil | 1.83 | ± | 1.02 |
| DMN plus soybean oil (LP) | 3.87 | ± | 1.93 |
| DMN plus soybean oil (HP) | 7.24 | ± | 3.23 |
| Methylmethanesulfonate (MMS) alone | 6.00 | ± | 1.30 |
| MMS plus coconut oil | 2.00 | ± | 1.04 |
| MMS plus soybean oil (LP) | 3.57 | ± | 0.93 |
| MMS plus soybean oil (HP) | 5.74 | ± | 1.37 |
| Tetracycline (Tet) alone | 8.93 | ± | 3.21 |
| Tet plus coconut oil | 1.70 | ± | 0.76 |
| Tet plus soybean oil (LP) | 4.70 | ± | 1.36 |
| Tet plus soybean oil (HP) | 6.48 | ± | 1.79 |

* LP = low peroxide value
** HP = high peroxide value

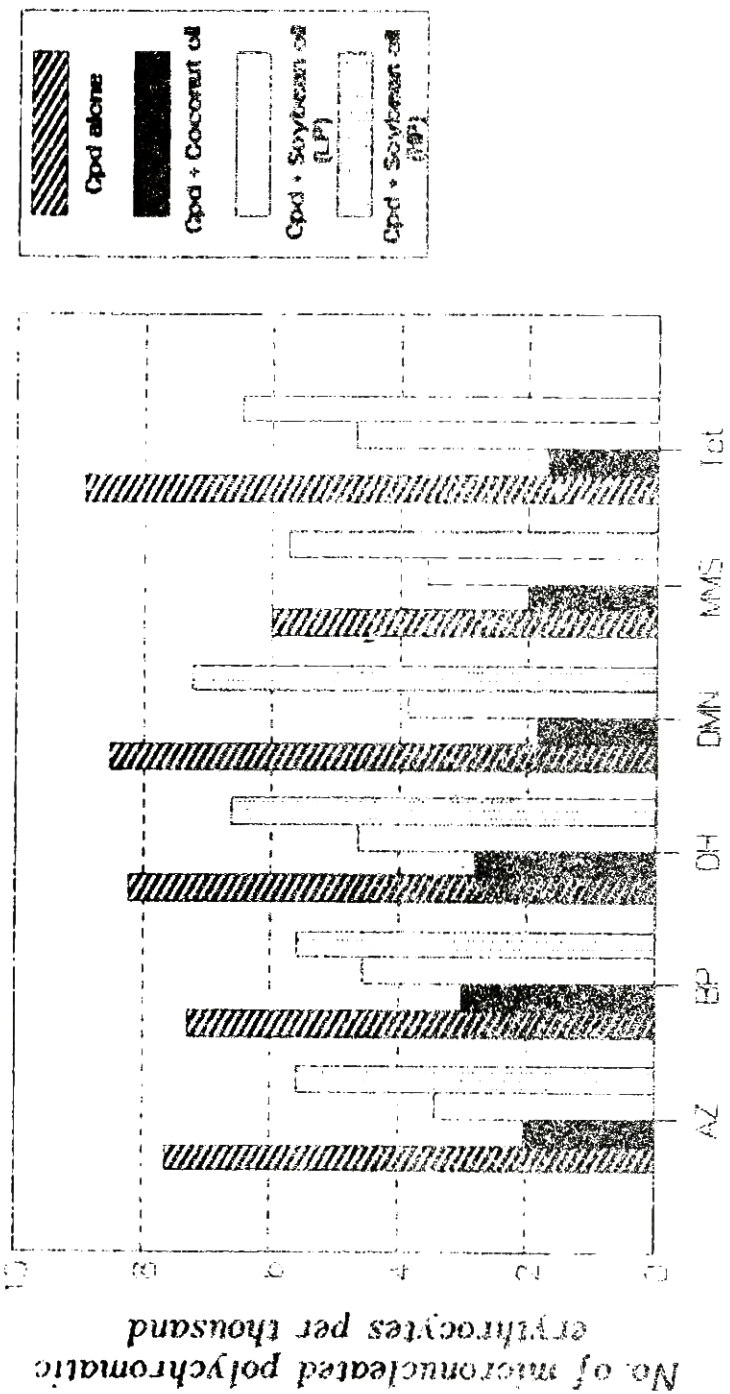


Figure 1. Effect of coconut oil and soybean oil on the genotoxicity of azaserine (AZ), benzo(a)pyrene (BP), dimethylhydrazine (DH), dimethylnitrosamine (DMN), methylmethanesulfonate (MMS) and tetracycline (Tet)

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