

ABSENCE OF GENOTOXIC EFFECTS OF CRUDE AND REFINED RED PALM OIL ON MOUSE BONE MARROW CELLS

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ABSTRACT

Red Palm Oil (RPO), extracted from fruits of *Elaeis guineensis*, is a complex mixture consisting of over 99% glycerides and about 1% non-glyceride compounds. Its orange-red colour is due to its high content of carotenoid pigments, mainly alpha- and beta-carotene. Based on the fact that palm oil is a rich source of provitamin A, and because it is largely consumed in North and Northeastern Brazil, we evaluated possible clastogenic and cytotoxic activities of this oil on mouse bone marrow cells *in vivo*, as well as the alpha- and beta-carotene content. The experiments were performed using samples of refined and crude palm oil, of which two different phases, supernatant, sediment, and the mixture of both, were tested. The animals were treated by gavage, at daily doses of 4.5 g/kg, for five consecutive days, and killed 24 hours after the last treatment, for chromosome preparations. The negative control group was treated with corn oil. There was no statistically significant difference in the frequency of chromosomal aberrations and in mitotic index when the animals which received palm oil were compared with the negative control. The beta-carotene content was higher than that of alpha-carotene, and the supernatant phase was the richest source of carotenoids. These findings suggest that RPO has no genotoxic effect.

INTRODUCTION

The palm tree (*Elaeis guineensis*) is originally from the West Coast of Africa, where from early times, the oil extracted from its fruit (mesocarp) has been an important source of fats and vitamins to the local diet. Today, red palm oil (RPO) is a major tropical product of great economic importance to a large number of developing countries and of considerable versatility within the edible oil industry (Clegg, 1973). In Brazil RPO is widely consumed, especially by populations in the North and Northeastern regions.

RPO is a complex mixture, consisting of over 99% glycerides and about 1% non-glyceride materials. The orange-red colour of RPO is due to its high content of carotenoid pigments, of which the major constituents are alpha- and beta-carotene, making this oil a rich source of provitamin A.

Although a diet with high fat and caloric content has been associated with elevated rates of various types of cancer as well as some cardiovascular diseases, preliminary nutritional and toxicological evaluation of RPO has demonstrated that this oil is nutritionally adequate and toxicologically safe for human consumption (Manorama *et al.*, 1989; Manorama and Rukmini, 1991a). However, few studies have been conducted to evaluate possible mutagenicity or carcinogenicity of this oil. Manorama *et al.* (1989), found no mutagenic effect of RPO when heated crude and refined palm oil were evaluated in an Ames bacterial assay system using *Salmonella typhimurium* strains TA100 and TA98, with metabolic activation (S9). A lower tumor promotion induced by palm oil was also observed by Sylvester *et al.* (1986), Sundram *et al.* (1989) and Nesaretnam *et al.* (1992) when compared with other edible oils. Azuine *et al.* (1992) demonstrated that a 1.5% palm oil diet totally abolished the tumorigenic effect induced by benzo[a]pyrene in the forestomach of mice.

Recently attention on cancer preventive qualities of naturally occurring compounds has been intensified and beta-carotene has been identified as a possible antimutagen and anticarcinogen. An inhibitory effect of palm carotene on glycocholic acid tumor promoting activity was reported by Okuzumi *et al.* (1992). Since RPO is a rich source of

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provitamin A and is widely consumed in Brazil, we were interested in investigating its possible mutagenicity (clastogenicity) and/or cytotoxicity, before evaluating its antimutagenic potential.

MATERIAL AND METHODS

The samples of crude RPO were obtained from the State of Bahia: Ilha de Itaparica (sample A) and Salvador (sample B) and the third sample, refined palm oil (sample C) was purchased from a local market (Salvador).

The quantitative determination of alpha- and beta-carotene was made using normal phase open-column chromatography, according to Rodriguez-Amaya (1989).

Mutagenicity tests were carried out with 8-10 week old Balb/C female mice (25-30 g) obtained from our own colony, maintained at 25°C, receiving food and water *ad libitum*.

Experimental groups were treated, by gavage, with two different phases of RPO, supernatant and sediment, and also, with a mixture of both, for five consecutive days (daily doses of 4.5 g/kg). The negative control group received just corn oil and, the positive control group received cyclophosphamide 20 mg/kg intra- peritoneally.

Each group consisted of 10 animals, which were killed, by cervical dislocation, 24 hours after the last treatment, the femurs from each animal were dissected and stripped clean of muscles, for cytological preparations from bone marrow (Hsu and Patton, 1969, modified by Zambrano *et al.*, 1982). The slides were stained in 10% aqueous Giemsa solution.

For each animal 100 bone marrow metaphase cells were analysed for evaluation of chromosome aberration and 1000 cells for determination of mitotic index. The types of chromosomal aberrations considered were chromatid and chromosome gaps, breaks, fragments, and exchanges.

The mitotic index was statistically analysed by the χ^2 test (Pereira, 1991), and the frequency of chromosome aberration was analysed using a conditional test based on an approximation to the Poisson distribution (Chakravarti *et al.*, 1967).

RESULTS

No statistically significant difference in the frequency of chromosomal aberrations and mitotic index was observed when treated groups with the RPO samples were compared with the control group (corn oil) (Table I).

Table I - Frequency of cells with chromosomal aberrations and mitotic index in mouse bone marrow cells after treatment with different phases of crude (Itaparica sample and Salvador sample) and refined (local market sample) red palm oil. One thousand cells from 10 animals were analyzed for each point.

Treatment	Types of chromatid aberration			Aberrant cells		Mitotic index %
	Gaps	Breaks	Fragments	No.	(%)	
<i>Itaparica sample</i>						
Corn oil ¹	3	0	4	7	(0.7)	2.18
Supernatant	3	1	2	6	(0.6)	2.26
Sediment	1	1	2	4	(0.4)	2.11
Supernatant plus sediment	3	1	2	6	(0.6)	2.04
<i>Salvador sample</i>						
Supernatant	5	3	2	10	(1.1)	2.24
Sediment	3	0	5	8	(0.8)	1.79
Supernatant plus sediment	4	1	4	9	(0.9)	2.23
<i>Local market sample</i>						
Supernatant	0	1	2	3	(0.3)	1.95
Sediment	2	0	4	6	(0.6)	2.08
Supernatant plus sediment	3	1	7	11	(1.1)	1.68
CPA ²	36	25	141	125	(12.5)*	1.50*

¹Negative control.

²CPA (cyclophosphamide - 20 mg/kg) - Positive control. More than one aberration/cell was found.

*Significant at 1% level, compared to negative control.

All aberrant cells scored presented only one type of chromosomal damage, the chromatid-type.

In samples A and B, beta-carotene content was much higher than alpha-carotene for all the different phases of the oil and, the supernatant phase had the highest range of carotenoid content (Table II). No statistically significant difference of alpha- and beta-carotene content was observed in the samples analysed.

Table II - Quantitative determination of alpha- and beta-carotene in different phases of crude red palm oil; Itaparica (A) and Salvador (B) samples.

Samples / Phases	Carotenoid contents ($\mu\text{g/g}$)	
	Alpha-carotene M \pm SD*	Beta-carotene M \pm SD*
A		
Supernatant	63.2 \pm 1.0	220.9 \pm 16.0
Supernatant plus sediment	24.6 \pm 3.6	76.8 \pm 1.4
B		
Supernatant	66.6 \pm 1.6	181.4 \pm 12.4
Sediment	29.8 \pm 1.9	44.4 \pm 1.8
Supernatant plus sediment	37.3 \pm 2.2	79.0 \pm 7.1

*Means and standard deviation of two determinations.

DISCUSSION

RPO, independent of origin and mode of production, did not induce a significant increase in the frequency of chromosomal aberrations in mouse bone marrow cells *in vivo*, after daily expositions of 4.5 g/kg. Furthermore, this dose did not promote any alteration in the mitotic index, suggesting that this oil has no cytotoxic effect.

The results obtained in the present study are in accordance with those obtained *in vitro* by Manorama *et al.* (1989), who demonstrated that crude palm oil as well as refined palm oil did not have any mutagenic effect, in *S. typhimurium*, even when heated up to 24 hours. According to these authors, when cooking fats are heated at a high temperature for a long time, several volatile and non-volatile oxidation products, some of them mutagens, are formed, increasing the mutagenic potential of continually re-used oil. However, the lack of mutagenic action observed may be due to the presence in RPO of high amounts of beta-carotene and other natural antioxidants (Manorama *et al.*, 1989).

Trujillo-Quijano *et al.* (1990), evaluating the carotenoid contents and vitamin A values in oils extracted

from three varieties of *Elaeis guineensis*, show that RPO is an important natural source of carotenoids with high provitamin A activity. Alpha- and beta-carotene contents obtained by these authors from fresh palm fruit oil, were higher than those obtained from the samples used in the present investigation. This difference could be attributed to the state of maturation of fruits used in each study, the variety of palm tree, the geographical and climatological circumstances of the cultivation, as well as the state of health and nutrition of the palm. However, in both studies, there was more beta- than alpha-carotene.

Due to the nutritional and economic advantages of RPO, there has been concern about the effects of this oil on human health. In India, for example, results obtained from studies with RPO have stimulated its use in supplementary feeding programmes for pre-school and school-going children, as a way of reducing vitamin A deficiency, one of the most urgent health problems in developing countries (Manorama and Rukmini, 1991b).

In North and Northeastern Brazil, the major RPO producing regions, there is a high incidence of diseases related to vitamin A deficiency. Thus, the possibility of increasing the consumption of RPO for these populations would be an important strategy for a better standard of life, since this oil did not show any toxicological and toxicogenetic effects and it is a natural source of provitamin A.

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RESUMO

O óleo de dendê, extraído de frutos da palmeira *Elaeis guineensis*, é uma mistura complexa que consiste de mais de 99% de glicérides e cerca de 1% de componentes secundários não-glicérides. A sua cor vermelho-alaranjada é devido aos seus altos níveis de carotenóides, principalmente alfa- e beta-caroteno. Baseado no fato de que o óleo de dendê é uma fonte rica em provitamina A, e no fato de que este óleo é amplamente consumido nas regiões norte e nordeste do Brasil, o presente estudo foi conduzido com o objetivo de avaliar um possível efeito mutagênico (clastogênico) e citotóxico deste óleo em células de medula óssea de camundongo *in vivo*, bem como o conteúdo de alfa- e beta-caroteno nas amostras testadas. Os experimentos foram realizados utilizando-se amostras de óleo de dendê bruto e refinado, das quais 2 fases foram testadas: sobrenadante ("flor do dendê") e sedimento e, ainda, a mistura de ambas. Os animais foram tratados por via oral, com doses diárias de 4,5 g/kg, durante 5 dias consecutivos, e sacrificados 24 horas após o último tratamento, para realização das

preparações citológicas. O grupo controle negativo foi tratado com óleo de milho e o controle positivo com ciclofosfamida (CPA) - 20 mg/kg. Os resultados obtidos através das análises citogenética e de citotoxicidade não mostraram diferenças estatisticamente significantes na frequência de aberrações cromossômicas e no índice mitótico, quando os grupos tratados com o óleo de dendê foram comparados com o controle negativo (óleo de milho). As determinações quantitativas de alfa- e beta-caroteno mostraram que os níveis de beta-caroteno foram mais altos que os de alfa-caroteno, para as 2 amostras testadas, e que a porção sobrenadante do óleo de dendê apresentou os mais altos níveis de carotenóides. Uma vez que, de acordo com estes resultados, o óleo de dendê não se mostrou genotóxico, novos estudos devem ser conduzidos no sentido de avaliar o potencial anticlastogênico deste óleo.

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