The Sciences 1 (3): 91-96
May-June, 2001

The Modulating Effects of Red Palm Oil (β-Carotene) on Aflatoxin β_1-induced Toxicity in Weaning rats

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Palm oil (β-Carotene) was evaluated for its ability to inhibit/ameliorate the aflatoxin β_1-induced toxicity in six groups of experimental rats thus (water control, aflatoxin β_1-treated, palm oil treated, palm oil and aflatoxin β_1-alternate group, palm oil and aflatoxin β_1 and aflatoxin β, and palm oil treated groups). Palm oil (1.4 μg β-carotene as Palm oil) was given orally while aflatoxin β_1 (2 mg kg^{-1} body weight) was given up to eight days and γ-glutamyl transferase (E. C. 2.3.2.2) activities were assayed in the liver and sera samples. The treatment with the palm oil caused a significant decrease in γ-glutamyl transferase activities in the palm oil treated groups compared with aflatoxin-treated controls in both liver and sera samples suggesting that palm oil contains antioxidant principles. Also treatment with palm oil ameliorated the histopathological lesion like fatty degeneration and necrosis induced by aflatoxin β_1 thus suggesting that palm oil was cytoprotective. It is concluded that pretreatment with palm oil was necessary for maximum inhibition of aflatoxin β_1-induced toxicity. The mechanism of inhibition by palm oil appeared to be inhibition of propagation of free radicals. Also administering palm oil and aflatoxin β_1 alternately appeared to be necessary for maximum inhibition of toxicity.

Key words: Red palm oil, aflatoxin, toxicity, weaning rats

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Introduction

Aflatoxin are secondary metabolites produced by the fungus, *Aspergillus flavus* that infects various agricultural root crops such as garri, yam, rice, groundnuts, sweet and irish potatoes (Adekunle, 1969; Maduka, 1993; Nwokolo, 1978). These foods and root crops occur in North Eastern zone of Nigeria as well as in the middle belt. The aflatoxin are characterised as β_1, β_2, G_1, G_2, M_1 and M_2 depending on their positions under the ultra violet light. Aflatoxin β_1 reported to be the most potent oral hepatocarcinogen, elaborated by *A. flavus* (Peers, 1967 and Wegan, 1967).

The metabolism of aflatoxin β_1 involves formation of a toxic intermediate 2,3-epoxide which is regarded as the ultimate carcinogen (Miller, 1974 and Oesch, 1973). And metabolic activation would appeared to be responsible for the numerous effects experienced during aflatoxin β_1 toxicity. Some of the toxic effects experienced with aflatoxin β_1 metabolism include ultrastructural effects, changes in RNA/DNA ratio, mitochondrial and template activities as well as inhibition of carbohydrate activities (Adekunle, 1969).

Epidemiological studies shown that the dietary factors such as β-carotene, vitamins A, E and selenium have true protective effects against cancer in humans (Burton, 1984). The report also established an inverse relationship between subjects treated with these dietary nutrients with cancer risks. In particular, β-carotene reported to trap free radicals (Krinsky, 1982), quench singlet oxygen species (Burton, 1984) and thus, reduce development of tumor under physiological conditions (Matthews Roth, 1982 and Seiffer, 1982) and reverse the early events in the development of carcinogenesis. β-carotene have a reducing effect on lipid peroxidation processes in the membrane. At higher pressures, it looses its antioxidant activity and exhibits an autocatalytic, pro-oxidant effects especially at relatively high concentrations. The functions of antioxidants in biological systems have been described (Matthews Roth, 1982; Seiffer, 1982 and Sugiyama, 1983). The primary antioxidant enzymes are superoxide dismutase, which dismutates superoxides radicals (O_2^-) to water and oxygen, catalase, which breaks down hydrogen peroxide to oxygen and water and glutathione, peroxidase, which breaks down hydrogen peroxides (Kumar, 1988). The antioxidants protect the cells against toxic damages by toxic radical ions. Vitamin E (α-tocopherol) also has the ability to break chains in the blood (Burton, 1984) and serves as a better antioxidants than β-carotene at higher pressures. The antioxidant property of α-tocopherol is enhanced by chroman head group with the heterocyclic ring optimizing the antioxidant property. Reports have shown that local dietary sources are rich in large amounts of antioxidant principles which will compare favourably with non antioxidants like vitamins E and C. For instance, *Sacoglottis gabonensis*, an alcoholic beverage additive and bergen in its active ingredient have been shown to protect against experimental membrane lipid peroxidation (Maduka, 1996). Similarly, later reports from the same laboratory showed that the bark extract of *S. gabonensis* and bergen in inhibited typical *in vitro* free radical reactions of carbon tetrachloride (CCl_4), doxorubicin. Some other natural products have exhibited cytoprotective effects against oxidants - induced hepatotoxicity, *Cyperus scariogus* extract protected male mice against acetaminophen and CCl_4- induced hepatotoxicity in male rats (Gilani, 1995). The presence of antioxidant principles in local natural sources such as vegetables, palm oil, carrots, natural stem and root bark extracts emphasises the need to further analyse these sources for cytoprotective properties. This will serves as a scientific basis to justify the continuous use of some of these extracts in folkloric medical practices.

The aim of research work to investigate the antioxidant property of palm oil on aflatoxin β_1-induced toxicity in the weaning rats. Aflatoxin is a major source of food spoilage in the same zone where palm oil is consumed. This communication, therefore, reports the effect of palm oil on aflatoxin β_1-induced toxicity in weaning rats, using γ-glutamyl transferase, a reliable index of various infiltrative hepatic disorders (Lum, 1972) as well as fatty degeneration (histopathological investigations) in livers and blood.

Materials and Methods

All the laboratory reagents used were of highest purity and of analytical grade. Kit for the assay of γ-glutamyl transferase was obtained from Sigma Chemical Company M.O. U.S.A. while palm oil was locally sourced from Orlu, Imo State. Pathogen free weaning rats of winter strain (150-200 g) obtained from the primate colony, Department of Biochemistry, University of Ibadan. They were stabilized for 2 days in a well ventilated room with 12 h. dark light^{-1} cycle and housed in plastic cages. They were given access of tap water ad libitum throughout the experimental period.

Table 1: The total dosages of β-carotene and aflatoxin β_1 administered per rat kg^{-1} body weight

Treatment	μg β-carotene per rat kg^{-1} body weight	mg aflatoxin β_1 per rat kg^{-1} body weight
General water control (group 1)	-	-
Palm oil treated control (group 2)	2.24	-
Aflatoxin β_1 treated control (group 3)	-	0.4
Palm oil and aflatoxin alternately (group 4)	0.84	1.40
Palm oil before aflatoxin β_1 treated (group 5)	1.96	0.40
Aflatoxin β_1 before palm oil treated (Group 1)	0.28	0.40

Treatment of animals and drug administration: After stabilization the rats were grouped into six experimental classes of five rats each and drugged as described below. The first group (water controls) were given nothing as drug but had unlimited drinking water for eight days. The second group palm oil- treated controls received oral administration of palm oil containing 1.4μg β-carotene daily for eight days. Rats in group three, the aflatoxin-treated controls, received 2 mg kg^{-1} body weight single i.p. administration of aflatoxin β_1 in $DMSO_4$ on the first day and thereafter had tap water as drinking water ad libidum until the last day of the experiment. Adenoid 1.4 μg β-carotene as palm oil was given orally to group four rats on the first day while 2 mg kg^{-1} body weight aflatoxin β_1 was administered on them as a single i.p. injection on the second day only. The palm oil and aflatoxin β_1 administration were repeated on the 3rd and 4th days respectively with a respective follow up on the 5th and 6th days respectively. Group five rats were given on a daily basis, and oral administration of palm oil as β-carotene (1.4μg β-carotene) for seven days and 2 mg kg^{-1} body weight aflatoxin β_1 intraperitoneally as a single administration on the eight day. In group six, the rats were each given 2 mg kg^{-1} body weight of aflatoxin β_1 intraperitoneally as single intraperitoneal administration in $DMSO_4$ on the first day and kept under observation until the eight day when they were given single oral administration of β-carotene (1.4 μg) as palm oil. The entire experiment was terminated on the night day when all the rats in groups 1 to 6 were sacrificed by cervical

dislocation and blood collected by cardiac puncture. The scheme of administration of the drugs are as shown on Table 1 and the design of the investigations was such as to make manifest any protective effect of palm oil on aflatoxin β_1-induced toxicity. Okoye and Neal (1991) reported that simultaneous and alternate administration of ethanol, aflatoxin β_1 and *sacoglottis gabonensis* bark extract an antioxidant were necessary to achieve alterations in DNA and albumin binding in rats. This observation is a toxic response and many study groups were therefore, created in order to observe the various effects of times of administration of aflatoxin β_1 and palm oil (β-carotene). β-carotene was estimated to be 1.4μg ml^{-1} palm oil by the method of Bassir (1965).

Tissue Homogenisation and Fractionation of Samples: Blood was collected into clean properly labelled sterile bottles according to groups and allowed to clot for 5 minutes. The blood collected were centrifuged at 3000 x g for 10 minutes at 4° C after which sera were recovered as the supernatant. The sera were used for determining the γ-glutamyl transferase activities.

Liver samples were excised, washed in ice-cold IM sucrose solution. They were then homogenised in IM sucrose solution (1.5 w/v) and centrifuged 3000 x g for ten minutes at 4°C. The supernatants were used for assaying γ-glutamyl transferase activities in all the treatment groups.

Liver slices were cut and fixed in 10% buffered formalin. Liver slices were prepared for microscopic examinations for histopathological studies following the method of Humason (1979). Histopathological slides were mounted under microscope at 400 x magnificant for evaluation of cytotoxicity in all the groups using fatty degeneration as the index for assessing toxicological risks and damages.

Assay Protocol: γ-glutamyl transferase activities (E.C. 2.3.2.2) were estimated spectrophotometrically at 405nm in the sera end liver homogenate samples following the procedure of Sigma Chemical Company, M.O., U.S.A. This was based on the rapid kinetic method of Scasz (1969) using γ-glutamyl -p-nitroanilide and glycylglycine as the donor and acceptor substrates respectively and results were presented as means ± S.D.Statistical comparisons were done between groups and within groups and the general water control using analysis of variance and confirmed by the Student's t-test.

Results

The results of the effect of palm oil on γ-glutamyl transferase activities in the sera and liver homogenates of all the rats after aflatoxin β_1-induced toxicity are as shown on Table 2.The mean γ-glutamyl transferase activities of aflatoxin β_1-treated control (group 3), palm oil before aflatoxin β_1 (group 5) and aflatoxin β_1 before palm oil treated rats (group 6) were significantly higher than the water control (group 1) enzyme levels. This suggested that treatment with aflatoxin β_1 and palm oil induced cytotoxicity in both liver and blood cells. When aflatoxin β_1 treated group and palm oil before aflatoxin β_1 (group 5) treated rats are compared, the aflatoxin β_1 treated control rats showed higher enzyme activities though not significantly. However, when the aflatoxin β_1 treated control enzyme activities were compared with aflatoxin β_1 before palm oil treated rats (group 6), it was found that the group 3 enzyme levels were significantly higher ($P < 0.05$) than the enzyme levels activities in group 6. This result showed that while the aflatoxin β_1-induced γ-glutamyl transferase activities, palm oil ameliorated the toxic effects of aflatoxin β_1 on the liver and blood cells. This showed that the antioxidant principles responsible for modulating aflatoxin β_1 are in the main contained in palm oil. Similarly, the enzyme activities of the control rats (group 1), palm oil treated (group 2) and alternate group (group 4) were comparable with no significant differences in the three groups in the liver homogenates. This same trend of result was essentially obtained in the sera samples for groups 2 and 4 except in group 1 control with a significantly elevated enzyme activity ($P < 0.05$), this suggested that palm oil contained principles that inhibited even normal metabolic/biological oxidation reactions due to normal cell metabolism. The liver γ-glutamyl activities were highest in group 5 (palm oil before aflatoxin β_1) and group 3 (aflatoxin β_1- control) rats thus confirming that aflatoxin β_1 is metabolism mostly in the liver. From the results it was also observed that administration of palm oil and aflatoxin β_1 was necessary for maximum inhibition/ protection by palm oil.

Table 2: The mean results of γ-glutamyl transferase activities of the serum and liver homogenates of the tests and control rats

Treatment	γ-glutamyl transferase activities (UL^{-1})	
	Liver	Serum
General water control (group 1)	10.61±00cef	18.85±6.17f
Palm oil treated control (group .2)	11.57±2.72cef	10.61±0.0ce
Aflatoxin β_1-treated control (group 3)	21.21±0.0bd	31.82±0.0d
Palm oil and aflatoxin β_1 alternately (group 4)	10.61±0.0cef	10.61±000ce
Palm oil before aflatoxin β_1 (group 5)	19.96±3.54bd	42.42±0.0b
Aflatoxin β_1 before Palm oil (group 6)	15.92±0.0a	21.21±0.0a

Results are means ± S.D of replicate determinations. Down a column, figures with different letters are statistically different ($p < 0.05$)

The results of the histopathological examinations of the livers of the groups are as shown on Figs.1, 2 and 3. As can be seen from the plates, the examinations did not show any tumor formations in any of the groups suggesting that the dose of aflatoxin β_1 administered was not enough to induce tumor formation or that the period of exposure was not long enough. However, when all the groups were assessed for fatty degeneration, the extent of damage was 85,70,80,60% for aflatoxin β_1-treated control (group 3), for palm oil and aflatoxin β_1 alternately (group 4), for palm oil before aflatoxin β_1 (group 5), treated rats and aflatoxin β_1 before palm oil (group 6), treated rats respectively. This confirms the observation made in the γ-glutamyl transferase activities that aflatoxin β_1 is an effective inducer of hepatotoxicity in rats. No serious lesions were seen in the livers of control (group 1) and palm oil treated rats (group 2) and suggesting that palm oil is not a hepatotoxicant. Also the connective tissues of hepatic blood vessels of groups 3, 4 and 5 rat livers were destroyed while there was only mild hypertrophy observed in group 6 rat livers. These observations have shown the extent of toxic damages by the aflatoxin β_1 administration and the degree of cytoprotection offered by palm oil. Necrosis was also observed in the livers of aflatoxin β_1 treated control livers thus confirming acute toxicity.

Discussion

Y-glutamyl transferase activities were evaluated in the liver homogenates and sera samples of weaning rates after administration of aflatoxin β_1, a hepatotoxicant and palm oil, a local dietary source rich in β-carotene.

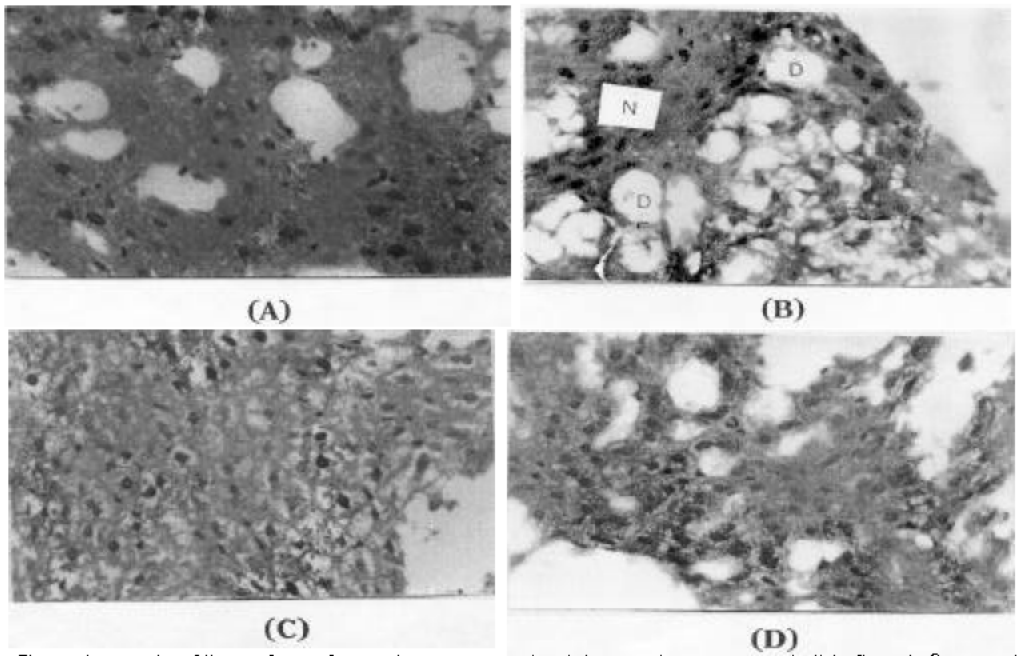

Fig. 1: Photomicrographs of livers of rats of controls as compared to (a) general water controls (b) aflatoxin β_1-treated (c) Palm oil treated (d) aflatoxin β_1 before palm oil treated. Note fatty degenerations (D) and necrosis (N) in aflatoxin β_1 treated control rats

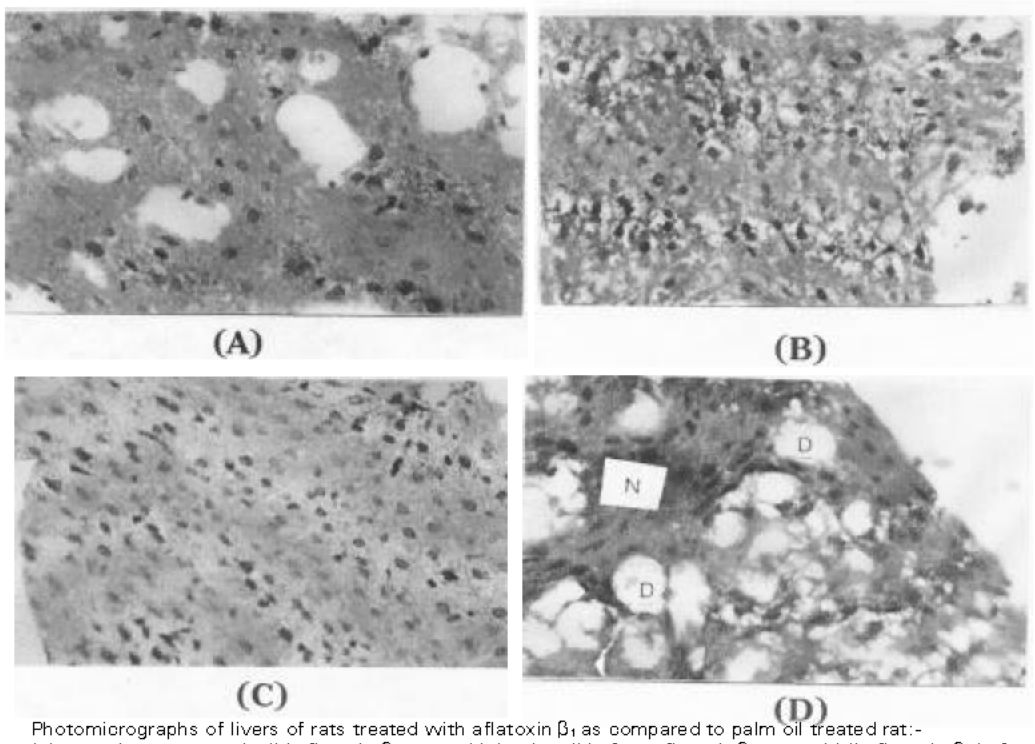

Fig. 2: Photomicrographs of livers of rats treated with aflatoxin β_1 as compared to palm oil treated rat:- (a) general water controls, (b) aflatoxin β_1-treated (c) palm oil before aflatoxin β_1 treated (d) aflatoxin β_1 before palm oil treated. Note fatty degeneration (D) and necrosis (N) in aflatoxin β_1 treated control rats and (c) palm oil before aflatoxin β_1 treatment group

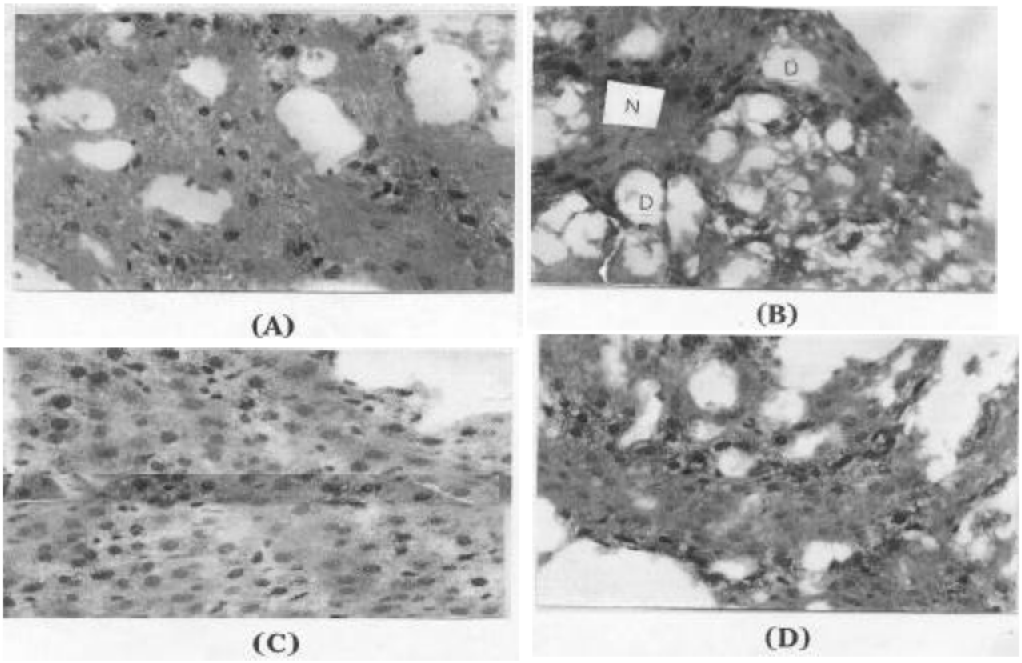

Fig. 3: Photomicrographs of livers of rats of controls as compared to (a) general water controls (b) palm oil treated (c) Palm oil and aflatoxin β_1 alternate group (d) aflatoxin β_1 treated controls. Note the fatty degenerations (D) in aflatoxin β_1 treated control rats and (c) Palm oil and aflatoxin β_1 alternate treatment groups due to administration of aflatoxin β1 and absence of the lesions in general controls and palm oil treated controls

Y-glutamyl transferase is a hepatotoxicity index (Knight, 1991) and a reliable marker enzyme in various infiltrative hepatic and obstructive disorders (Lum, 1972).The series of investigating reported were to ascertain if palm oil could exhibit anti-toxic properties against aflatoxin β_1-induced toxicity. The statistically significant result obtained between the various treatment groups and the controls were due to the design of the experiment while treating the rats with the drugs, aflatoxin β_1 and palm oil.

Experimental results showed that palm oil inhibited the γ-glutamyl transferase activities significantly ($P < 0.05$) when palm oil treated rats were compared the palm oil free test rats given aflatoxin β_1.The aflatoxin β_1 induced the enzyme activities markedly (compare groups 3 and 1) while palm oil inhibited the enzyme activities. This set of results clearly demonstrates that palm oil possesses antioxidant properties against aflatoxin β_1 in weaning rats and also favour a conclusion that administration of palm oil after aflatoxin β_1 was necessary for the palm oil to clearly and maximally inhibit elevation in enzyme activities. This result corroborates a similar earlier report of Okoye and Neal (1991) where simultaneous administration of aflatoxin β_1, *Sacoglottis gabonensis* stem bark extract and alcohol was necessary to alter the DNA and albumin binding in rats. This indirect evidence of antioxidant property was later confirmed by (Maduka, 1993). The results of the histopathological examinations essentially followed the same trend as was observed with γ-glutamyl transferase activities. Fatty degeneration was absolute while necrosis was also observed in livers of aflatoxin β_1 treated rats thus confirming the hepatotoxic nature of aflatoxin β_1. Histopathological examinations are used to corroborate the results of biochemical parameters in the assessment of toxicological risks and damages by xenobiotics (Zimmerman, 1982). However all the lesions observed in aflatoxin β_1-intoxicated groups were mostly inhibited in groups given palm oil and absent in the general water control and palm oil treated control rats. It was observed that inspite of the large quantity of palm oil administered in group 5 the extent of fatty degeneration appeared not to be significantly different when groups 5 and 3 rats are compared. This suggests an earlier observation that administration of an antioxidant like palm oil (β-carotene) is necessary after aflatoxin β_1 toxicity.

The palm oil possesses an antioxidant properties against aflatoxin β_1 by inhibiting free radical driven lesions in the liver and also by inhibiting the induction of γ-glutamyl transferase activities in the liver and blood. It is a reliable tumor marker and other hepatotoxic damages. (Sigma Chemical Company, U.S.A., 1989; Scasz; Knight 1981). β-carotene was reported to materially reduce human cancer rates, in particular various retinals can reversibly suppress the malignant behaviour of cultural cells that have been transformed by viruses, chemicals of ionizing radiation (Peto, 1981) or even can delay or prevent the onset of cancer in experimental animals that had been treated before. Results support that β-carotene is one of the major components of palm oil responsible for the observed antioxidant action. The structure of β-carotene resembles that described for α tocopherol (Burton, 1984) in which the chroman head group and systematic conjugation of double bonds along the heterocyclic ring enhance antioxidant properties.

Since β-carotene & α tocopherol are of the radical trapping

mechanism, results o btained favoure the conclusion that palm oil traps free radicals and most likely reduces the rate of chain of propagation. However, because of the presence of long chain polyunsaturated fat ty acids in palm oil which can induce lipid peroxidation, caution need to be exercised in extrapolating the antioxidant potentials or efficacious of the oil.

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