The Modulating Effects of Red Palm Oil (β-Carotene) on Aflatoxin β₁-induced Toxicity in Weanling Rats

H. C. C. Maduka, A. O. Uwaifo and J. O. Nwankwo

Palm oil (β-Carotene) was evaluated for its ability to inhibit/ameliorate the aflatoxin β₁-induced toxicity in six groups of experimental rats thus (water control, aflatoxin β₁-treated, palm oil treated, palm oil and aflatoxin β₁-alternate group, palm oil and aflatoxin β₁, and palm oil treated groups). Palm oil (1.4 μg β-carotene as Palm oil) was given orally while aflatoxin β₁ (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 β₁, thus suggesting that palm oil was cytoprotective. It is concluded that pretreatment with palm oil was necessary for maximum inhibition of aflatoxin β₁-induced toxicity. The mechanism of inhibition by palm oil appeared to be inhibition of propagation of free radicals. Also administering palm oil and aflatoxin β₁ alternately appeared to be necessary for maximum inhibition of toxicity.

Key words: Red palm oil, aflatoxin, toxicity, weanling rats
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**Introduction**

Aflatoxins are secondary metabolites produced by the fungus, *Aspergillus flavus* that affects various agricultural root crops such as garlic, yam, rice, guestnuts, sweet and Irish potatoes (Adedunke, 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 aflatoxins are characterised as β1, β2, G1, G2, M1, and M2 depending on their positions under the ultraviolet light. Aflatoxin β1 is reported to be the most potent of the hepatocarcinogen, elaborated by A. flavus (Peers, 1887 and Wigan, 1967).

The metabolism of aflatoxin β1 involves formation of a toxic intermediate 2,3-epoxide which is regarded as the ultimate carcinogen (Miller, 1974 and Oeser, 1973). And metabolic activation would appear to be responsible for the numerous effects experienced during aflatoxin β1 toxicity. Some of the toxic effects experienced with aflatoxin β1 metabolism include ultrastructural effects, changes in RNA/DNA ratio, mitochondrial and template activities as well as inhibition of carbohydrate activities (Adedunke, 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 Seifler, 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; Seifler, 1982 and Sugiyama, 1983). The primary antioxidant enzymes are superoxide dismutase, which dismutates superoxides radicals (O2.) 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 radicals 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 chromon 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, *Sagittifolius gabonensis*, an alcoholic beverage additive and benrger 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 benrger in inhibited typical in vitro free radical reactions of carbon tetrachloride (CCl4), doxorubicin. Some other natural products have exhibited cytoprotective effects against oxidants - induced hepatoxotoxicity. *Cyperus** racialis extract protected male mice against acetaminophen and COCl2 induced hepatotoxicity in male rats (Gilani, 1996).

The presence of antioxidant principles in local natural sources such as vegetables, palm oil, carotols, natural stem and root bark extracts emphasizes 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 β1-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 β1-induced toxicity in weaning rats, using y-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 y-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 baskan. They were stabilized for 2 days in a well ventilated room with 12 h. dark light 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 β1 administered per rat kg body weight**

<table>
<thead>
<tr>
<th>Treatment</th>
<th>µg β-carotene per rat kg body weight</th>
<th>mg aflatoxin β1 per rat kg body weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>General water control (group 1)</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>Palm oil-treated control (group 2)</td>
<td>2.24</td>
<td>-</td>
</tr>
<tr>
<td>Aflatoxin β1-treated control (group 3)</td>
<td>-</td>
<td>0.4</td>
</tr>
<tr>
<td>Palm oil and aflatoxin administered (group 4)</td>
<td>0.64</td>
<td>1.40</td>
</tr>
<tr>
<td>Palm oil before aflatoxin β1 treated (group 5)</td>
<td>1.56</td>
<td>0.40</td>
</tr>
<tr>
<td>Aflatoxin β1 before palm oil treated (group 1)</td>
<td>0.28</td>
<td>0.40</td>
</tr>
</tbody>
</table>

**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 body weight single i.p administration of aflatoxin β1 in CSM50 on the first day and thereafter had tap water as drinking water ad libitum 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 body weight aflatoxin β1 was administered on them as a single i.p injection on the second day only. The palm oil and aflatoxin β1 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 body weight aflatoxin β1, intraperitoneally as single administration on the eighth day. In group six, the rats were each given 2 mg kg body weight of aflatoxin β1, intraperitoneally as single intraperitoneal administration in DMSO on the first day and kept under observation until the eighth 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 5 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- induced toxicity. Okoye and Neil (1991) reported that simultaneous and alternate administration of ethanol, aflatoxin β, and saccharin was effective in ameliorating the toxic effects of aflatoxin β, and aflatoxin β. Saccharin was estimated to be 1.4 μg/ml of palm oil by the method of Blass (1965).

Tissue Homogenisation and Fractionation of Samples: Blood was collected in clean properly labeled 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 at 3000 x g for 10 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 examination for histopathological studies following the method of Humason (1979). Histopathological slides were mounted under microscope at 400 x magnification for evaluation of cytotoxicity in all the groups using fatty degeneration as the index for assessing toxicological risks and damages.

Assay Protocols γ-glutamyl transferase activities (E.C. 2.3.2.2) were estimated spectrophotometrically at 405nm in the sera and liver homogenates samples following the procedure of Sigma Chemical Company, M.O., U.S.A. This was based on the rapid kinetic method of Scas (1969) using γ-glutamyl-p-nitroanilide and glycylglycine as the donor and acceptor substrates respectively and results were presented as means ± S.D. Statistically 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-induced toxicity are as shown on Table 2. The mean γ-glutamyl transferase activities of aflatoxin β-treated control (group 1), palm oil before aflatoxin β (group 2) and after aflatoxin β, 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, and palm oil induced cytotoxicity in both liver and blood cells. When aflatoxin β, treated group and palm oil before aflatoxin β (group B) treated rats were compared, the aflatoxin β, treated control rats showed higher enzyme activities though not significantly. However, when the aflatoxin β, treated control enzyme activities were compared with aflatoxin β, before palm oil treated rats (group 6), it was found that the group 3 enzyme levels were significantly higher (P<0.05) than the corresponding activities in group 6. This result showed while the aflatoxin β, induced γ-glutamyl transferase activities, palm oil ameliorated the toxic effects of aflatoxin β, on the liver and blood cells. This showed the antioxidant principles responsible for modulating aflatoxin β, 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) and group 3 (aflatoxin β control) rats thus confirming that aflatoxin β is metabolism mostly in the liver. From the results it was also observed that administration of palm oil and aflatoxin β, was necessary for maximum inhibition/ protection by palm oil.

Assay Protocols γ-glutamyl transferase activities of the serum and liver homogenates of the tests and control rats

<table>
<thead>
<tr>
<th>Treatment</th>
<th>γ-glutamyl transferase activities (U/L)</th>
</tr>
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<tbody>
<tr>
<td>General water control</td>
<td>10.61 ± 0.00ef</td>
</tr>
<tr>
<td>(group 1)</td>
<td></td>
</tr>
<tr>
<td>Palm oil treated control</td>
<td>11.57 ± 2.72cef</td>
</tr>
<tr>
<td>(group 2)</td>
<td></td>
</tr>
<tr>
<td>Aflatoxin β, treated control</td>
<td>21.21 ± 0.06bd</td>
</tr>
<tr>
<td>(group 3)</td>
<td></td>
</tr>
<tr>
<td>Palm oil and aflatoxin β, alternately (group 4)</td>
<td>10.61 ± 0.00ef</td>
</tr>
<tr>
<td>Palm oil before aflatoxin β, (group 5)</td>
<td>19.95 ± 3.54bc</td>
</tr>
<tr>
<td>Aflatoxin β, before Palm oil (group 6)</td>
<td>15.92 ± 0.0a</td>
</tr>
</tbody>
</table>

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 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 88, 70, 80, 60% for aflatoxin β, treated control (group 3), for palm oil and aflatoxin β, alternately (group 4), for palm oil before aflatoxin β, (group 5), treated rats and aflatoxin β, before palm oil (group 6), treated rats respectively. This confirms the observation made in the γ-glutamyl transferase activities that aflatoxin β, 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 rats livers were destroyed while there was only mild hyper trophy observed in group 6 rat livers. These observations have shown the extent of toxic damages by the aflatoxin β, administration and the degree of cytotoxicity offered by palm oil. Necrosis was also observed in the livers of aflatoxin β, treated control livers thus confirming acute toxicity.

Discussion γ-glutamyl transferase activities were evaluated in the liver homogenates and sera samples of weaning rats after administration of aflatoxin β, 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, (b) aflatoxin B1-treated, (c) palm oil treated (d) aflatoxin B1 before palm oil treated. Note fatty degeneration (D) and necrosis (N) in aflatoxin B1 treated control rats.

Fig. 2: Photomicrographs of livers of rats treated with aflatoxin B1 as compared to palm oil treated rats: (a) general water controls, (b) aflatoxin B1-treated (c) palm oil before aflatoxin B1 treated (d) aflatoxin B1 before palm oil treated. Note fatty degeneration (D) and necrosis (N) in aflatoxin B1 treated control rats and (e) palm oil before aflatoxin B1 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 $\beta_1$ alternate group (d) aflatoxin $\beta_1$ treated controls. Note the fatty degenerations (C) in aflatoxin $\beta_1$ treated control rats and (c) Palm oil and aflatoxin $\beta_1$ alternate treatment groups due to administration of aflatoxin $\beta_1$ and absence of the lesions in general controls and palm oil treated controls.

$\gamma$-glutamyl transferase is a hepatotoxicity index (Knight, 1981) and a reliable marker enzyme in various in vitro and obstructive disorders (Lum, 1972). The series of investigations reported were to ascertain if palm oil could exhibit antitoxic properties against aflatoxin $\beta_1$-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 $\beta_1$ and palm oil. Experimental results showed that palm oil inhibited the $\gamma$-glutamyl transferase activities significantly ($P < 0.05$) when palm oil treated rats were compared the palm oil free test rats given aflatoxin $\beta_1$. The aflatoxin $\beta_1$ 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 antioxidative properties against aflatoxin $\beta_1$, in weaning rats and also favour a conclusion that administration of palm oil after aflatoxin $\beta_1$ 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 (1981) where simultaneous administration of aflatoxin $\beta_1$, Sceoglottis gaborunzase stem bark extract and alcohol was necessary to alter the DNA and albumin binding in rats. The indirect evidence of antioxidant property was later confirmed by (Maduka, 1985). The results of the histopathological examinations essentially followed the same trend as was observed with $\gamma$-glutamyl transferase activities. Fatty degeneration was absolute while necrosis was also observed in livers of aflatoxin $\beta_1$ treated rats thus confirming the hepatotoxic nature of aflatoxin $\beta_1$. Histopathological examinations are used to corroborate the results of biochemical parameters in the assessment of toxicological risks and damages by xenobiotics (Zimmerman, 1882). However all the lesions observed in aflatoxin $\beta_1$- 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 intake of the large quantity of palm oil administered in group 6 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 ($\beta$-carotene) is necessary after aflatoxin $\beta_1$ toxicity. The palm oil possesses an antioxidative properties against aflatoxin $\beta_1$ by inhibiting free radical driven lesions in the liver and also by inhibiting the induction of $\gamma$-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; Sauer, Knight 1981). $\beta$-Carotene was reported to materially reduce human cancer rates in particular various rats have been treated with $\beta$-carotene which could not be observed with controls. The structure of $\beta$-carotene resembles that described for tocopherols (Burton, 1984) in which the chroman head group and systematics conjugation of double bonds along the heterocyclic ring enhance antioxidant properties. Since $\beta$-carotene & $\alpha$-tocopherol are of the radical trapping.
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mechanism, results obtained favor 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 fatty acids in palm oil which can induce lipid peroxidation, caution need to be exercised in extrapolating the antioxidant potentials or efficacious of the oil.

References