AFRICAN JOURNAL OF MEDICINE and medical sciences

VOLUME 29, NUMBER 2, JUNE 2000

EDITOR:
B. O. OSOTIMEHIN

ASSISTANT EDITOR:
A. O. UWAIFO

ISSN 1116 — 4077

Afr. J. Med. med. Sci. (2000) **29**, 127-132

Modulation of carbon tetrachloride-induced lipid peroxidation and xenobiotic-metabolizing enzymes in rats fed browned yam flour diet

EO Farombi, JO Nwankwo and GO Emerole
Department of Biochemistry, Drug Metabolism and Toxicology Unit, College of Medicine, University of Ibadan, Nigeria.

Summary
The modulatory effect of browned yam flour diet, a dietary staple in south-western Nigeria, on carbon tetrachloride (CCl_4)-mediated lipid peroxidation and on the activities of liver microsomal and cytosolic enzymes was studied in male rats. Browned yam flour diet fed at the level of 25% and 50% to rats for 5 weeks significantly reduced the lipid peroxidation induced by CCl_4 (0.5 ml/kg/wk) administered two weeks after starting the animals with the diets. The diets elicited 62% and 79% reductions in CCl_4-mediated peroxidation, respectively, in the absence of exogenously added oxidants. The activities of microsomal aniline hydroxylase (AH), aminopyrine-*N*-demethylase (APD), pentoxyresorufin-*O*-dealkylase (PROD) and cytosolic GSH *S*-transferase (GST) were increased when rats were fed the 25% or 50% browned yam flour diets. Browned yam flour fed at the level of 25% to rats decreased the CCl_4-mediated reduction in the activities of microsomal AH, APD, PROD and GST by 64%, 28%, 58% and 25%, respectively, and by 82%, 48%, 83% and 55% when rats were fed with 50% of the diet. The results suggest that browned yam flour diet could protect against chemically-mediated lipid peroxidation and tissue damage possibly by scavenging chemically generated reactive species and enhancing carcinogen-detoxifying system.

Keywords: *Browned yam flour, lipid peroxidation, CCl_4, xenobiotic-metabolizing enzymes, browning reaction Products.*

Résumé
L'effet modulatrice de lalimentation a la poudre marrow d'ignane, un met principale du sud-ouest du Nigeria sur la peroxydation des lipes par le carton tetrachloride (CCC4) est Nir l'activile des enzymes microsomale et cytosolie du foie avait ete etudie chez les rats male. Les rats nourrit avec un regime alimentaire coupose de 25% et 50% d'igname marron pendant 5 femaines avaient une activile significativement reduite de peroudation des lipides par le CCC4 (0,5 ml/kg/femaine) administre l semaines apres le commencement du regime alimentaire. Les regemes avaine tinduient 62% et 79% de ereduction de la peroxidation des lipides induite par le CCC4 respectivement en absence des oxidante exogenes. Les activites de l'aniline hydroxylase (AH) des microsome, aminopyrine -N-demethylase (APD), pento xyresorufin -O-dealkylose (PROD) et de la GSHS transferase (GST avaient augmente chez les rats nourient avec 25% un 50% de poadre d'igname marron.Les rats nourrient avec 25% de poudre d'igname avaient reduit les reductions un medier par la CCC4 dans le AH microsomal, APD, PROD et GST de 64%, 28%, 58% et 25% respectivement et de 82%, 48%, 83% et 55% l'orsque les rats etaient nourrient avec 50% de poudre d'igname marron. Ces resultats suggerent que liigame la poudre d-igname marron pourrait protégé contre la peroxydation des lipides induits par des substances chimiques et les endomagement des tissues probablement en eliminant les na especos reactives et en augmentatant le systeme de detoxification des carcinogenes.

Correspondence: Dr. E.O. Farombi, Department of Biochemistry, Drug Metabolism and Toxicology Unit, College of Medicine, University of Ibadan, Ibadan, Nigeria.

Introduction

It is known that a number of chemical compounds mediate toxicity by metabolism into free radicals, which start-off the process of lipid peroxidation in cell membranes. Such radicals target thiol groups, enzymes, amino acids, nucleotides and unsaturated fatty acids in the cell [1]. The effect of these radicals in the presence of oxygen on polyunsaturated fatty acid chains results in lipid peroxidation [2,3]

Carbon tetrachloride (CCl_4), perhaps the most-studied liver toxicant, is activated by hepatic microsomal mixed function oxidase to the trichloromethyl radical ($CCl^{\cdot}_3$) and in the presence of oxygen, trichloromethyl peroxy radical ($CCl_3O^{\cdot}_2$) which is more reactive. $CCl_3O^{\cdot}_2$ causes peroxidation of polyunsaturated fatty acids, while $CCl^{\cdot}_3$ is more relevant in covalent binding to both lipid and protein components of the membrane [4,5]. Xenobiotic-metabolising enzymes are responsible for the generation and detoxification of radical and reactive metabolites and alterations in the activities of these enzymes may affect the potency of reactive cytotoxic radical and electrophilic metabolites generated in the cells [6,7].

Various dietary components, naturally occurring or synthetics have, been shown to protect against experimental carcinogenesis. Among these components, dietary flavonoids have been shown to protect against toxicity induced by compounds such as CCl_4 [8]. Several studies have related the chemoprotective activities of flavonoids to their ability to inhibit peroxidative damage caused by environmental compounds [8]. Also the modulation of the activities of xenobiotic-metabolising enzymes such as glutathione *S*-transferase, aminopyrine-*N*-demethylase, aniline hydroxylase and pentoxyresorufin-*O*-dealkylase by these minor dietary constituents is well documented [9,10].

In recent times, foods containing brown-colored compounds formed from enzymatic and non-enzymatic browning reactions have been shown to play a significant role in the modification of cellular damage mediated by carcinogens. The modulation of drug metabolizing enzymes [11] and lipid peroxidation [12] by

browning reaction products have been reported. Yam, widely cultivated in the tropics contains polyphenols such as catechins, epicatechins, chlorogenic acids, leucoanthocyanidins and anthocyanins [13,14]. It is known that polyphenolic compounds in yam undergo polyphenolic oxidase-catalysed reactions to form o-quinones, their primary oxidation products, which react with other components to form brown polymeric compounds [15,16,17]. Also, amino acids and proteins in the yam, when heated, can react non-enzymatically with sugars forming brown-coloured compounds commonly called Maillard reaction products [18]. These reactions are responsible for the brown colour associated with browned yam flour diet, a staple commonly consumed in West Africa especially in the southern parts of Nigeria [15].

In our earlier report, we demonstrated for the first time that browned yam flour diet (fed at the level of 25% to rats) could modulate the toxicity mediated by some chemical compounds including CCl_4 [19]. However, the mechanisms by which the diet protects against the toxicity of foreign compounds are unknown. The study reported here was, therefore, conducted to determine the effect of browned yam flour diet on CCl_4-induced lipid peroxidation and on xenobiotic-metabolizing enzymes in rat liver in order to understand the mechanisms by which the diet elicits protective effects on chemically induced toxicity.

Materials and methods

Chemical

CCl_4 was purchased from Hopkin & Williams, Chadwell Heath, Essex, UK and distilled before use. Bovine serum albumin was purchased from Sigma Chem. Co., London, UK. Aniline hydrochloride, p-aminopyrine, pentoxyresorufin, resorufin, 1,2-dichloro-4-nitrobenzene (CDNB), NADP, glucose 6-phosphate, glucose 6-phosphate dehydrogenase, trichloroacetic acid (TCA) and N-2 hydroxyethyl piperazine N-ethane sulphonic acid (HEPES) were purchased from Sigma Chem. Co. (St Louis, MO, USA). Semicarbazide was purchased from Fluka AG, Chemische Fabrik, CH-9470, Buchs, Switzerland. Thiobarbituric acid (TBA) and ethylene diamine tetraacetic acid (EDTA) were purchased from Sigma Chem. Co., London, UK. All other reagents were of analytical grade and were purchased from British Drug Houses, Poole, England.

Animals and diets

Yams (*Dioscorea rotundata*) grown in Ibadan, Nigeria and harvested were dried and processed into yam flour which was turned brown by the addition of hot water. The resulting paste (browned yam flour) was dried in an oven at 50°C for 12hr. Browned yam flour diet was substituted at 25% or 50% of the basal diet (Table 1) so that both were isocaloric and isonitrogenous according to Hedin & Adachi [20] and as reported previously [19]. In the calculation, maize and groundnut cakes were varied while the other components in the diets were fixed. Overall crude protein content and total metabolisable energy were calculated from the protein and energy values of the food components [20]. The diets were pelleted and fed to the animals.

Animals and treatments

Male weanling albino rats (Wistar strain) about 4 weeks old, weighing 85-94 g, obtained from the primate colony, Biochemistry Department, University of Ibadan, Nigeria were fed and watered *ad lib* in wire-mesh cages on a 12hr light/dark cycles for 5 weeks. Thirty-five animals were divided into seven groups 1, 2, 3, 4, 5, 6 and 7. Rats in groups 1 (control), 3 and 7 were fed the basal diet and those in groups 2 & 4, and 5 & 6 were placed on 25% and 50% browned yam flour diet, respectively, for 5 weeks. Two weeks after the start of the experiment, rats in groups 3, 4 and 6 were treated orally with CCl_4 (0.5 ml/kg body wt) once a week for 3 weeks. Group 7 served as positive control and seven days to the end of the experiment, 0.1% Phenobarbital (PB) was mixed with drinking water. Food consumption, weight gain and liver weight were recorded for all animals. Wire-mesh cages used to house the animals coupled with standardized feeding containers minimized food spillage and allowed proper record of food consumption.

Table 1: Composition of basal and browned yam flour containing diets

Component	Basal diet	Percentage of component in Brown yam flour diet 25%	50%
Maize	51.65	23.09	3.71
Groundnut cake (GNC)	34.03	37.60	34.98
Non-nutritive fibre	5	5	2
Corn oil	5	5	5
*Vitamin-mineral premix	1	1	1
Oyster shell	1	1	1
Bone meal	2	2	2
Methionine	0.3	0.3	0.3
Sodium chloride	0.25	0.25	0.25
Browned yam flour	0.0	25	50

Crude protein of all diets: approximately 20%
Metabolizable energy of all diets: approximately 3.11 cal/g
Total lipid of maize and browned yam flour: approximately 3%
*Composition of vitamin-mineral premix per 6 mg; Vit.B6, 0.6 mg; Vit. B12, 0.004 mg; Nicotinic acid, 12 mg; Folic acid, 0.4 mg; Choline chloride, 125 mg; Iron, 18 mg; Copper, 1 mg; Manganese, 24 mg; Cobalt, 0.2 mg; Zinc, 20 mg; Iodine, 0.8 g Vit.A, 4000 IU; Vit. D3, 800 IU; Vit E, 4 mg; Vit. K, 3.081; Vit. B1, 0.60 mg; Vit. B2, 2 mg; Ca-pantothenate, 3. mg; Selenium, 0.008 mg; Sorbic acid, 0.3 mg.

Preparation of microsomal and cytosolic fractions

The rats were sacrificed by cervical dislocation and livers were quickly removed, washed in 1.15% KCl, dried, weighed and homogenised in 4 vol. of isotonic phosphate buffer, pH 7.4 and centrifuged at 9,000 g for 20 minutes to obtain the post mitochondria supernatant fraction. Microsomes were pelleted at 100,000 g for 90 minutes by subsequent centrifugation. The supernatant (cytosolic fraction) was immediately frozen on dry ice. Microsomes were resuspended in 0.25M sucrose solution. Aliquots of this suspension were stored at –80°C and thawed before use. All procedures were carried out at temperatures between 0-4°C.

Protein determination

Microsomal and cytosolic protein concentrations were determined according to the method of Lowry et al. [21] using bovine serum albumin as standard

CCl_4-induced lipid peroxidation

Lipid peroxidation was assayed by measuring the thiobarbituric acid reactive (TBAR) products using the procedure of Walls et al. [22]. About 1 mg/ml of liver microsomal suspension in isotonic phosphate buffer, pH 7.4 was incubated for 6 hr at 37°C in a shaking water bath with or without 0.1 mM $FeSO_4$, 1 mM EDTA, 1 mM ascorbate and 0.2 mM H_2O_2 (final concentrations). 0.5 ml of 0.75% thiobarbituric acid (TBA) in 0.1 M HCl was added to 0.5 ml of incubation mixture already quenched with 0.5 ml of 10% TCA. The mixture was heated at 90-95°C for 20 minutes and after cooling centrifuged for 10 minutes at 780 g. The supernatant was transferred into acid resistant tubes and centrifuged at 32,000 g for 10 minutes. The absorbance of the resulting clear solution was determined at 532 nm with phosphate buffer as blank.

Enzyme assays

Incubation mixture for the assay of aniline hydroxylase, aminopyrine-*N*-demethylase and pentoxyresorufin-*O*-dealkylase activities contained 2.5 µmol Mg^{2+} as NADPH generating system and 1 mg/ml microsomal fraction and 14 µmol aniline hydrochloride or 10 µmol aminopyrine or 10 nmol pentoxyresorufin in a total volume of 2 ml.

Aniline hydroxylase activity (AH)

This was assayed by the spectrophotometric method of *Schenkman et al.* [23] by measuring p- aminophenol formed from aniline hydrochloride. Incubation was for 20 minutes at 37°C and pH 7.5.

Aminopyrine-N-demethylase activity (APD)

This was assayed by the method of *LaDu et al.* [24] Incubation was for 10 minutes at 37°C at pH 7.5. The estimation of the amount of formaldehyde formed during *N*-demethylation of aminopyrine was carried out by the method of Nash [25].

Pentoxyresorufin-O-dealkylase activity (PROD)

Microsomal pentoxyresorufin-*O*-dealkylase was estimated by the method of Guo et al. [26]. Incubation was for 2 minutes at 37°C in the presence of 100 mM HEPES (pH 7.8) and the reaction was terminated by the addition of methanol (2 ml). The fluorescence of resorufin formed during the *O*-dealkylation of pentoxyresorufin was measured with (λex 550 and λex 585 nm using Perkin-Elmer fluorescence spectrophotometer 204.

Glutathione-S-transferase activity (GST)

Cytosolic glutathione *S*-transferase activity was determined by the method of Habig et al. [27] using 1,2-dichloro-4-nitro benzene (CDNB) as substrate. Incubation mixture described previously [28] contained 50 µl of CDNB, 10 µl 0.1 M reduced glutathione, 0.93 ml 0.1 M phosphate buffer (pH 6.5) and 10 µl cytosolic fraction in a total volume of 1 ml. The change in absorbance at 340 was followed for 60 seconds in Perkin-Elmer spectrophotometer 548 at 30°C.

Statistics

The data were analyzed by a two-tailed Student's t-test. P values less than 0.05 were considered statistically significant

Results

As shown in Table 2 there was no significant change in peroxidation between the animals fed browned yam flour diet and the control rats both in the presence and absence of oxidants. The animals that were treated with CCl_4 (group 3) however produced significant increase ($P < 0.05$) in lipid peroxidation when compared with control animals. The values obtained in the presence of oxidants were significantly higher than the ones obtained without the oxidants. Browned yam flour fed at the levels of 25% and 50% significantly reduced the level of peroxidation induced by CCl_4 (62% and 79% reductions, respectively) when no oxidants were added to the incubation medium ($P < 0.05$) (Table 2).

Table 2: Effect of 25% and 50% browned yam flour diet on CCl_4-induced microsomal lipid peroxidation in the rat.

		Lipid peroxidation (TBARS) (A532 nm/mg protein)	
Component		Without oxidants	With oxidants
1.	Control	0.34 ± 0.1	0.56 ±0.03j
2.	25% B.Y.F.	0.33 ± 0.1	0.55 ± 0.02j
3.	CCl_4	0.65 ± 0.03*	0.85 ± 0.1j
4.	CCl_4 + 25% B.Y.F.	0.44 ± 0.01**	0.65 ± 0.03j
5.	50% B.Y.F.	0.34 ± 0.1	0.56 ± 0.02j
6.	CCl_4 + 50%B.Y.F.	0.38 ± 0.02***	0.60 ± 0.03j

The results are the means ± S.D for 5 rats in each group.

*' Exogenously generated oxidants (1 mM $FeSO_4$, 1 mM EDTA, 1 mM Ascorbate and 0.2 mM H_2O_2). Significantly different from controls *$P < 0.001$, from chemical (CCl_4) **$P < 0.001$, from control and group 4 ***$P < 0.001$. Significantly different from corresponding values (without oxidants) †$P < 0.05$. B.Y.F = Browned Yam Flour.*

Table 3 shows that there were no significant differences ($P > 0.05$) in final body weight, weight gain and feed consumption for animals in groups 1, 2, 5 and 7. However, there was a significant decrease in CCl_4 treatment group (group3) for the parameters compared with the control. There was no significant difference in feed efficiency for animals in all the groups. Compared to the control, browned yam flour, CCl_4 and phenobarbital groups had significantly higher terminal and relative liver weights. The results further indicate that the diet may not alter the nutritional status of the animals since it did not affect significantly the nutritional parameters recorded.

As seen in Table 4, browned yam flour fed at the level of 25% significantly increased the activities of AH, APD, PROD and GST (1.7, 1.2, 1.2 and 1.2 fold, respectively) over the control; while 50% browned yam flour diet elicited increases (2.4, 1.3, 2.3 and 1.3 fold, respectively) in the activities of these enzymes (Table 4). The Phenobarbital group showed a highly significant increase

Table 3: Effect of 25% and 50% browned yam flour diet on body weight, weight gain, feed efficiency and relative liver weight of rats following oral pretreatment with 0.5 ml CCl_4/kg body weight.

Group	Final Weight (g)	Weight gain (g)	Feed intake (g)	Feed efficiency	Terminal liver wt (g)	As % of body weight
1. Control	179 ± 15.7	82 ± 9.3	251± 7.0	0.3 ± 0.09	5.5 ± 0.2	3.1 ±0.1
2. 25% B.Y.F	173 ±12.5	88 ± 6.3	250 ± 8.0	0.3 ± 0.05	6.0 ± 0.1H	3.5±0.2H
3. CCl_4	149 ± 5.0*	55 ± 4.9*	200 ± 2.0*	0.3 ± 0.05	8.0 + 0.5*	5.4 + 0.3*
4. CCl_4+ 25% B.Y.F	166 ± 4.0**	73 ± 3.0**	240 ± 7.3**	0.3 ± 0.03	6.5 ± 0.2**	3.9 ± 0.3**
5. 50% B.Y.F	176 ± 11.2	85 ± 7.9	247 ± 8.0	0.3 ± 0.03	7.3 ± 0.3I	4.1 ± 0.2I
6. CCl_4+ 50% B.Y.F	174 ±4***	80 ± 4***	245 ± 6.0***	0.3 ± 0.03	6.0 ± 0.3***	3.4 ± 0.1***
7. PB	162 ± 10.0	72 ± 7.8	236 ± 9.0	0.3 ± 0.05	9.2 ± 0.3#	5.7 ± 0.2#

The results are the means ± S.D for 5 rats in each group. Feed efficiency = weight gained/feed intake.
** Significantly different from control, P < 0.001*
*** Significantly different from group 3, P < p.001*
****Significantly different from group 4, P< 0.05*
†Significantly different from control, P< 0.001
‡Significantly different from control
#Significantly from control, P< 0.001

Table 4: Effect of 25% and 50% browned yam flour diets on the activities of microsomal aniline hydroxylase, aminopyrine-*N*-demethylase, pentoxyresorufin-*O*-dealkylase and cytosolic glutathione *S*-transferase following oral pretreatment with 0.5 ml CCl_4/ kg body weight.

Group	Enzyme activity (nmol product formed/min/mg protein) Aniline hydroxylase	*p*-aminopyrine *N*-demethylase	Pentoxyresorufin *O*-dealkylase	Cytosolic GST
1. Control	1.1 ± 0.2	5.0 ± 0.4	1.2 ± 0.2	670 ± 22.2
2. 25% B.Y.F	1.9 ± 0.1	5.8 ± 0.2*	1.5 ± 0.1*	780 ± 21.2*
3. CCl_4	0.2 ± 0.1**	2.6 ± 0.3**	0.1 ± 0.1**	362 ± 20.4**
4. CCl_4 + 25% B.Y.F	0.9 ± 0.1***	4.0 ± 0.5***	0.8 ± 0.2***	532 ± 22.1***
5. 50% B.Y.F	2.6 ± 0.1H	6.5 ± 0.2H	2.8 ± 0.1H	900 ± 20.2H
6. CCl_4+ 50% B.Y.F	1.1 ± 0.1I	5.0 ± 0.3I	1.1 ± 0.1I	732 ± 22.1I
7. PB	3.8 ± 0.1#	7.1 ± 0.1 #	3.5 ± 0.2#	1012 ± 22.3#

*The results are the means ± S.D for 5 rats in each group. * Significantly different from control, P< 0.05*
***Significantly different from control, P< 0.001. ***Significantly different from group 3, P< 0.001. †Significantly different from control and group 2 P < 0.05. ‡Significantly different from groups 3, P< 0.001 and 4, P<0.05. #Significantly different control P<0.001.*

($P < 0.001$) in the activities of all the enzymes compared with control (3.5, 1.4, 2.9 and 1.5 fold, respectively, for AH, APD, PROD and GST).

The results revealed a significant reduction ($P < 0.001$) in the activities of the microsomal enzymes: aniline hydroxylase, aminopyrine-*N*-demethylase, pentoxyresorufin-*O*-dealkylase and cytosolic GSH-*S*-transferase within the period of exposure of rats to 0.5 ml/kg of CCl_4 compared with control animals (Table 4). The results further show that feeding rats with 25% browned yam flour diet decreased significantly the CCl_4 mediated reduction in the activities of AH, APD, PROD and GST by 64%, 28%, 58% and 25%, respectively, and by 82%, 48%, 83% and 55% when rats were fed with 50% browned yam flour diet.

Discussion

The apparent lipid peroxidation caused by CCl_4 intoxication as indicated by a significant increase in the formation of TBARS, in the absence of exogenously added oxidants, confirms earlier investigations [1,29,30]. The increase in the value of peroxidation products in the presence of exogenously generated reactive oxygen species is attributed to the well established fact that iron could catalyze the generation of toxic reactive oxygen [3,31]. It has also been reported that ascorbate may act as prooxidant [32].

Our findings show a significant reduction in lipid peroxidation caused by CCl_4 when rats were fed the 25% and 50% browned yam flour diets. The results further show a greater effectiveness of the latter diet compared with the former. The eduction in lipid peroxidation observed in this study by the diets may be interpreted to mean that browned yam flour can scavenge the reactive intermediates generated by CCl_4 intoxication that would otherwise lead to lipid peroxidation and cell necrosis. Recent studies have indicated the antioxidative and scavenging effect of browning reaction products [12]. This may explain the basis of protection offered by the diet against hepatotoxicity induced by CCl_4 and other compounds observed in investigations carried out in our laboratory [19,33].

The significant reduction in the activities of AH, APD, PROD and GST following exposure of rats to CCl_4 is in agreement with the reports of previous workers that pretreatment of animals with this compound destroys the activities of mixed function oxidases probably by the reactive species which it produces [34,35]. The apparent protection of liver enzymes from CCl_4-induced toxicity by 25% and 50% (more effective) browned yam flour diets as evidenced by modulation of the reduction in the activities of these enzymes, suggests that the diet could possibly act as an antioxidant and protect against toxicity mediated by chemically generated reactive species.

The increase in relative liver weight (expressed as % of body weight) in animals fed browned yam flour diet indicates that they may have had an increased capacity through feeding on the diet to metabolize xenobiotic substances via enzyme induction. Previous studies have shown that induction of monooxygenase and/or conjugating enzymes could be a mechanism by which dietary components protect against carcinogenic insult [6,7,9,36]. A prominent feature of these enzymatic inductions is an increase in GSH *S*-transferase activity [37,38]. Thus induction of GSH *S*-transferase activity observed in this study by browned yam flour diet and probably other conjugating enzymes which were not assayed in the present investigation suggests that the diet might offer protection against the toxicity of foreign compounds by inducing increases in carcinogen detoxification system. Furthermore, the overall inductive effect on both phases I and II systems in aggregate had been reported by Wattenberg [6] to result in enhanced carcinogen detoxification.

The results of this study indicate that browned yam flour could protect against chemically induced tissue damage by an antioxidative action and an inductive effect on carcinogen detoxifying system.

Acknowledgements

This work was supported by grants from Cadbury (Nig) PLC and the Wellcome Trust (Nig) to E.O.F. We also thank Dr. G. Thottappilly of International Institute of Tropical Agriculture (I.I.T.A), Ibadan, Nigeria for making facilities available for enzyme studies.

References

1. Shertzer HG, Berger ML and Tabor MW. Intervention in free radical mediated hepatotoxicity and lipid peroxidation by indole-3-carbinol. Biochem Pharmacol 1988; 37: 333-338.
2. Cerutti P. Prooxidant states and tumor promotion. Science 1985; 227: 375-380.
3. Rice-Evans C. Oxidative modifications in erythrocytes induced by iron in free radicals, oxidant stress and drug action Edited by Rice-Evans C. (Richeliu Press, London), 1987; pp.307-330.
4. Cheesman KH. In: Free radicals, lipid peroxidation and cancer, edited by McBrien D.C.H., Slater T.F. (Academic Press London, 1982; pp. 197-202.
5. Cholbi MR, Paya M and Alcaraz MJ. Inhibitory effect of phenolic compounds on CCl4-induced microsomal lipid peroxidation: Experientia 1991; 47: 195-199.
6. Wattenberg LW. Inhibition of carcinogenesis by minor dietary constituents. Cancer Res1983; 52: 2085s-2091s.
7. Whitty JP and Bjeldane LF. The effect of dietary cabbage on xenobiotic-metabolizing enzymes and binding of aflatoxin B_1 to hepatic DNA in rats. Food Chemical Toxicol 1987; 25: 581-587.
8. Colbi HD, Brogan III WC and Miles PR. Carbon tetrachloride-induced changes in adrenal microsomal mixed-function oxidases and lipid peroxidation. Toxicol Appl Pharmacol 1981; 60: 492-499.
9. Brousad C, Siess MH, Vernevaut MF and Suschetet M. Comparison of the effect of feeding quercetin or flavone on hepatic and intestinal drug-metabolizing enzymes of the rat. Food Chem Toxicol 1988; 26: 99-103.
10. Siess MH, Giullermic M, Bon AN and Suschetet M. Induction of monooxygenase and transferase activities in rat by dietary administration of flavonoids. Xenobiotica 1989;19: 1379-1386.
11. Kitts DD and Wu CH Powrie WD. Effect of glucose lysine Maillard reaction-product fractions on tissue xenobiotic enzyme-systems. J Agric Food Chem 1993; 41: 2359-2363.
12. Yen GC and Hsieh PP. Antioxidative activity and scavenging effects on active oxygen of xylose-lysine Maillard reaction-products. J Sci Food Agric 1995; 67: 415-420
13. Imbert MP and Seaforth C . Anthocyanins in Dioscorea alata L. Experientia 1968; 24: 445-447.
14. Martin FW, Ruberte R. The polyphenol of D. alata (yam) tubers associated with oxidative browning. J

Agric Food Chem1975; 24: 67-70.

15. Ozo ON. Analytical procedure for polyphenols and poplyphenol oxidase of yam tubers. In: Advances in Yam Research: The Biochemistry and Technology of Yam tuber, edited by G. Osuji (AI-United Industries and Shipping Inc Taiwan and Frontline publishers, Enugu, Nigeria) 1985; pp. 165-213.
16. Walker JRL. Enzymic browning in foods. Enz Technol Dig 1975; 4: 89-100.
17. Mayer AM and Harel E. Polyphenol oxidase in plants. Phytochemistry 1979;18: 193-215.
18. Maillard LC, Action des acides amine sur les scres. Formation de melanoidines par voie methodique. Comptes Rendus hebdmadaires des Seances de l" Academie des Science 1912; 154: 66.
19. Farombi EO, Nwankwo JO and Emerole GO. Possible modulatory effect of browned yam flour diet on chemically-induced toxicity in the rat. Food Chem Toxicol 1997; 35: 975-979.
20. Hedin PA and Adachi RA. Effects of diet and time of feeding on gastrointestinal gas production in rats. J Nutr 1962; 77: 229-236.
21. Lowry OH, Rosebrough NJ, Farr AL and Randall RJ. Protein measurement with the Folin phenol reagent. J Biol Chem 1951; 193: 26-275.
22. Walls R, Kumar KS and Hochstein P. Ageing of human erythrocytes. Arch Biochem Biophys 1976; 100: 119-128
23. Schenkman JB, Remmer H and Estabrook JW. Spectral studies of drug interaction with hepatic microsomal cytochrome. Mol Pharmacol 1967; 3: 113-123.
24. La Du BN, Gaudettel Trousof N and Brodie BB. Enzymatic dealkylation of aminopyrine and other alkyamines. J Biol Sci 1955; 214: 741-752.
25. Nash T. The colorimetric estimation of formaldehyde by means of the Hantzsech reaction. Biochem J 1953; 55: 416-421.
26. Guo Z, Smith TJ, Wang E, Sadrieh N, Ma Q, Thomas PE and Yang CS. Effect of phenethyl isothiocyanate, a carcinogenic inhibitor, on xenobiotic metabolizing enzymes and nitrosamine metabolism in rats. Carcinogenesis 1992; 13: 2205-2210.
27. Habig WH, Pabst MJ and Jakoby WB. Glutathione-*S*-transferases. The first enzymatic step in mercapturic acid formation. J Biol Chem 1974; 349: 7130-7139.
28. Emerole GO and Thabrew MI. Changes in some hepatic microsomal components induced by prolong administration of chloroquine. Biochem Pharma 983; 32: 3003-3009.
29. Rao KA and Recknagel RO. Early onset lipoperoxidation in rat liver after carbon tetrachlori administration. Exp Mol Path 1968; 9: 271-278.
30. Glende EA, Hruszkewycz AM and Recknagel R Critical role of lipid peroxidation in cart tetrachloride-induced loss of aminopyrine-demethylase, cytochrome P450 and glucose phosphatase. Biochem. Pharmacol. 1976; 25: 21 2167.
31. Aust SD, Morehouse LA and Thomas CE Role metals in oxygen radical reactions. J. Free Rad E Med 1985; 1: 3-25.
32. Roemer KG, Kamal AM and Freundt KJ. In or directed toxicity edited by brown S.S. (Pergan Press, New York)1981; pp. 68-73.
33. Farombi EO. Modulation of hepatic xenobio metabolizing enzymes by browned yam flour die rats fed bromobenzene. Proc Fed Afr Soc Bioch Mol Biol 1998; 1: 11-14.
34. Thabrew MI, Emerole GO and Subbarao VV. Effec Liv. 52 on carbon tetrachloride-induced changes hepatic microsomal drug metabolizing enzymes of rat. Toxicol Lett 1982; 14: 183-188.
35. Colbi H D, Brogan III WC and Miles PR. Carl tetrachloride-induced changes is adrenal microso mixed-function oxidases and lipid peroxidati Toxicol Appl Pharmacol. 1981; 60: 492-499.
36. Nixon JE, Hendricks JD, Pawloski NE, Pereira (Sinnhuber RO and Bailey GS Inhibition of aflato B_1 carcinogenesis in rainbow trout by flavone indole compounds. Carcinogenesis 1984;55: 625-61
37. Benson AM, Batzinger RP, Ou SL, Bueding E, (YN and Talalay P. Elevation of hepatic glutathione transferase activities and protection against mutage metabolites by dietary antioxidants. Cancer Res 19 12: 4486-4495.
38. Benson AM, Cha YN, Bueding E, Heine HS a Talalay P. Elevation of extrahepatic glutathione transferase and epoxide hydratase activities by 2 (tert-butyl-4-hydroxyanisole. Cancer Res 1979; 2971-2977.