Comparative Effects of Scopoletin and Aflatoxin B_1 on Bovine Hepatic Mitochondrial Respiration *in vitro*

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A comparative study of the *in vitro* effects of the coumarin compounds, scopoletin and aflatoxin B_1 (AFB_1), on bovine (*Bos indicus*) hepatic mitochondrial respiration was carried out polarographically, using isocitrate -NAD^+ (3 – site), succinate (2- site), and reduced cytochrome *c* (1 – site), as respiratory substrates. Both scopoletin and AFB_1 elicited a substrate – dependent stimulation or inhibition of the mitochondrial states 4 and 3 respiration. The results suggest that AFB_1 has a higher tendency to inhibit the mitochondrial respiration than scopoletin, while scopoletin showed higher uncoupling effects than AFB_1. The effects of scopoletin and AFB_1 on mitochondria were more pronounced on the electron transport than on phosphorylation reaction. The extent (3–35%) of AFB_1 induced inhibition of bovine mitochondrial respiration observed in this study, was appreciably lower than the values indicated in other animal species (rats and guinea fowls) reported in previous studies using equivalent concentrations of the toxin. These results were discussed in terms of the susceptibility of the animal species to the toxic effects of scopoletin and AFB_1.

Introduction

The aetiology of many chronic and degenerative disease processes in animal systems are greatly influenced by non nutrient contaminants of foods (Werther, 1980; Boyd *et al.*, 1982). Aflatoxin B_1 (AFB_1), is a known food contaminant and a good number of carcinogenic and non carcinogenic disease processes have been associated with it (Detroy *et al.*, 1971; Heathcote and Hibbert, 1978; WHO, 1979). Scopoletin (6 – methoxy – 7 – hydroxycoumarin) on the other hand, is a non-nutrient constituent of foods particularly cassava products (Obidoa and Obasi, 1991) and potatoes (Minamikawa *et al.*, 1983). Recent studies from our laboratories (Obasi *et al.*, 1994, 1996) have shown that scopoletin elicited alterations in some liver functions of chicks and guinea pigs.

Both scopoletin and AFB_1 have been shown to be retained in the human body when taken through diet (Hendrickse *et al.*, 1983; Obasi and Obidoa, 1995). Preliminary observations from our laboratories have also indicated traces of AFB_1 in the liver and muscle samples collected from cows slaughtered in our locality (unpublished laboratory notes). The bovine species could be exposed to both scopoletin and AFB_1 through diet (Uzoho, 1991) and hence could be affected by the toxic manifestations of these compounds.

Although the alteration of liver mitochondrial activities in a number of laboratory animal species by aflatoxins (particularly AFB_1) have been reported (Newberne and Butler, 1969; Pai *et al.*, 1975; Obidoa and Siddiqui, 1978), information regarding the effects of AFB_1 on the liver mitochondrial respiration of bovine species has been lacking. In addition, there has been a dearth of information on the effects of scopoletin on liver mitochondrial respiration in animal species including the bovine species.

The objective of the present study is to compare the effects of both scopoletin and AFB_1 on the bovine liver mitochondrial respiratory activities. The investigation would also, further extend the frontiers for understanding the influence of species variations in the *in vitro* toxic effects of AFB_1.

Materials and Methods

Chemicals and Reagents

The common reagents and chemicals used in these experiments were of analytical grade, the

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highest purity available, and obtained from Sigma Co (St Louis, U. S.A), BDH (Poole, England), or Merck (Darmstadt, Germany). The purity of both scopoletin (Serva Fein Biochemica Heidelberg) and AFB_1 (Sigma) was in each case, determined by the production of a single bluish fluorescent (uv-short wavelength) spot when the compound was subjected to thin layer chromatography using methanol in chloroform (3:97 v/v) as the development solvent (Obasi and Obidoa, 1994).

Isolation of bovine hepatic mitochondria

Liver samples (approximately 20 g) were freshly obtained from male bovine (*Bos indicus*) animals slaughtered at the local abattoir. The liver samples (collected within 10 minutes after decapitating the animal) were placed in ice-cold 0.34 M sucrose. After peeling off the outer coat (liver pulp) of the liver, weighed fractions of the samples were minced and washed with 0.34 M sucrose solution. Mitochondria were subsequently isolated from washed liver slices following the procedures described by Johnson and Lardy (1967), using 0.34 M sucrose as isolation medium. Isolated mitochondria were suspended in 0.34 M sucrose -0.02 M Tris (hydroxymethyl) aminomethane – HCl buffer (pH 7.4). Mitochondria were isolated within one hour of liver collection and used within five hours of the isolation. Mitochondrial protein was determined by the biuret method (Thorne, 1984).

Measurement of oxygen uptake (respiration)

Mitochondrial oxygen uptake was measured by the polarographic method employing the Clark's oxygen electrode as described by Olorunsogo *et al.* (1990). The oxygen uptake was recorded with a model 53 oxygen monitor carrying a YSI 5331 oxygen probe. The oxygen electrode solution adopted in this experiment was that described by Obidoa and Ngodo (1984). It contained in a final volume of 3 ml, 150 mM sucrose; 10 mM potassium phosphate buffer pH 7.4; 5 mM $MgCl_2$; 20 mM KCl, and approximately 0.3 mg of mitochondiral protein. State 1 to state 4 respiratory transition was induced by adding to the electrode solution (reaction medium), 30 µl aliquot of the substrates consisting of either isocitrate/NAD^+ (2 mM/100 µM), or sodium succinate (2 mM) or (ferro) cytochrome *c* (0.4 mM). The amounts in brackets represent the final concentrations of the respective compounds in the reaction media (final volume was 3.0 ml). Reduced cytochrome *c* was prepared by the reaction of sodium dithionate with cytochrome *c* (horse type 3, Sigma). State 4 to state 3 transition was by the addition of 10 µM ADP (disodium salt). Respiratory control ratio (RCR) was calculated from the expression:

$$RCR = \frac{O_2 \text{ Uptake in the presence of substrate + ADP (State 3)}}{O_2 \text{ Uptake in the presence of substrate only (State 4).}}$$

The amount of oxygen taken up by the mitochondria at the various respiratory states were calculated as described by Eastabrook (1967). In the experiment with 2,4 dinitrophenol (DNP), aliquots of the compound (final concentration in medium was 40 µM) was added to the reaction medium prior to the addition of mitochondria. Both scopoletin and AFB_1 solution in 10% N,N^1 dimethyl formamide (DMF)were added as 0.1 ml aliquots to provide the final concentrations in the reaction medium. Control experiments were performed by addition of 0.1 ml of 10% DMF solution, (the solvent for both scopoletin and AFB_1) instead of the toxin solutions.

Measurement of Ca^{2+} uptake:

Measurement of Ca^{2+} uptake was based on the principle that mitochondrial Ca^{2+} induced swelling is related to:
(i uptake of Ca^{2+} by mitochondria and;
(ii uncoupling of oxidative phosphorylation (Lehninger *et al.*, 1967). The changes in the absorbance at 520 nm (Milton Roy Spectronic 1201) of mitochondria (0.1 mg protein) suspended in 2.5 ml of the reaction medium (34 mM sucrose -20 mM Tris-HCl pH 7.4) containing 1.32 mM Ca^{2+} were determined at 2 minutes intervals for 8 minutes. Percentage Ca^{2+} -induced swelling was calculated from the difference between the mitochondrial absorbance at zero time and at the end of 8 minutes. The percentage Ca^{2+} -induced swelling was calculated from the experiments carried out in the presence of scopoletin and AFB_1 as shown in Table IV. Control experiments were carried out by addition of 0.1 ml 10% DMF solution instead of either scopoletin or AFB_1 solutions.

Analysis

All statistical analyses were by students t – test.

Results

Effects of scopoletin and AFB_1 on state 4 respiration

The calculated amounts of oxygen uptake in the absence (control) and presence of different concentrations of either scopoletin or AFB_1 at different respiratory states of bovine liver mitochondria are shown in Tables I–III.

The results in Table I indicate that relative to control, AFB_1 at 5–20 nmol/mg mitochondrial protein (mmp) concentrations, elicited a concentration dependent inhibition (11–33%) of isocitrate -NAD^+ (3 – site) dependent state 4 respiration. On the other hand, while the 5–10 nmol/mmp concentrations of scopoletin showed a stimulation (11–22%) of the same respiration, 20 nmol/mmp concentration of scopoletin inhibited it by 22%. The effects of AFB_1 were significant ($P < 0.05$) at 20 nmol/mmp while scopoletin showed significant ($P < 0.05$) effects at 10 nmol/mmp concentrations (stimulation) and at 20 nmol/mmp concentrations (inhibition).

Table II shows that at 5–10 nmol/mmp concentrations, AFB_1 significantly stimulated (25%) succinate dependent (2 – site) state 4 respiration. At 20 nmol/mmp concentration however, AFB_1 had

Table I. Effects of scopoletin and aflatoxin B_1 on oxygen uptake of bovine hepatic mitochondrial isocitrate – NAD^+ dependent respiration.

* Concentration of compound (per mg mitochondrial protein)	State 4	State 3	Respiratory control ratio (RCR)	State 4 plus 2.4 DNP
Control (10% DMF)	28.08 ± 0.32	115.44 ± 3.20	4.10	30.25 ± 0.31
5 nmol AFB_1	24.96 ± 0.41	#143.52 ± 2.50	#5.75	#23.69 ± 0.52
10 nmol AFB_1	24.76 ±0.20	127.92 ± 1.64	#5.17	#22.30 ± 0.50
20 nmol AFB_1	+#18.71 ± 0.37	121.68 ± 1.32	#6.50	#16.47 ± 0.54
5 nmol Scopoletin	31.20 ± 0.84	@#165.36 ± 1.48	#5.30	#35.02 ± 0.22
10 nmol Scopoletin	#34.32 ± 0.54	@#171.60 ± 3.11	#5.00	#37.84 ± 0.71
20 nmol Scopoletin	#21.84 ± 0.56	@#134.16 ± 2.08	#5.38	#20.73 ± 0.95

States 4 and 3. Oxygen uptake were measured as nmol O_2/min x mg mitochondrial protein using the methods indicated. Values presented represent mean ± S.D of six replicate data pooled from three different mitochondrial preparations.
* Values of concentration represent final concentrations of the respective compounds in the reaction media (final volume of media was 3.0 ml).
# Statistically different from control values ($P < 0.05$).
+ Inhibitory effect of AFB_1 significantly higher than corresponding scopoletin values ($P < 0.05$).
@ Stimulatory effect of scopoletin significantly higher than that of the corresponding AFB_1 values ($P < 0.05$).

Table II. Effects of scopoletin and aflatoxin B_1 on oxygen uptake of bovine hepatic mitochondrial succinate dependent respiration.

* Concentration of compound (per mg mitochondrial protein)	State 4	State 3	Respiratory control ratio (RCR)	State 4 plus 2.4 DNP
Control (10% DMF)	12.48 ± 0.58	106.08 ± 1.15	8.05	14.88 ± 1.01
5 nmol AFB_1	#15.60 ± 0.61	102.96 ± 1.20	#6.60	14.60 ± 0.44
10 nmol AFB_1	#15.70 ± 0.71	93.60 ± 1.0	#5.96	15.05 ± 0.68
20 nmol AFB_1	12.41 ± 0.25	+#68.64 ± 2.41	#5.50	14.90 ± 0.56
5 nmol Scopoletin	@#18.72 ± 0.85	#124.80 ± 2.35	#6.67	#20.25 ± 0.72
10 nmol Scopoletin	@#18.62 ± 0.75	115.44 ± 1.63	#6.20	#21.32 ± 0.31
20 nmol Scopoletin	#18.52 ± 0.65	96.72 ± 2.25	#5.22	#22.10 ± 0.69

State 4 and 3. Oxygen uptake were measured as nmol O_2/min x mg mitochondrial protein as described in Table I legend. Values presented represent mean ± S. D. of six replicate data pooled from three different mitochondrial preparations. All table signs are as described in Table I.

no significant ($P > 0.05$) effect on the succinate dependent state 4 respiration. Scopoletin at 5–20 nmol/mmp concentrations significantly stimulated (~ 50%) the succinate dependent state 4 respiration.

When reduced (ferro) cytochrome *c* (1 – site) dependent state 4 respiration was considered, it was observed (Table III), that none of the three concentrations of AFB_1 tested had any observable effect on the state 4 respiration, whereas scopoletin at 5–10 nmol/mmp stimulated the respiration by 75% which then fell to 25%, at a rate inversely related to its concentration. The respiration was inhibited by 20 nmol/mmp concentrations of scopoletin.

Effects of scopoletin and AFB_1 on ADP – stimulated (state 3) respiration

The oxygen uptake during ATP stimulated (active or state 3) respiration of the mitochondria in the presence of the different substrates are also presented in the tables. AFB_1 at 5–20 nmol/mmp enhanced the isocitrate – NAD^+ dependent state 3 respiration (Table I) of the mitochondria by 5–25%, while scopoletin at equivalent concentrations also stimulated the respiration by 16–49%. However, while only the effects of 5 nmol/mmp concentration of AFB_1 was significant, the effects of all the concentrations of scopoletin (5–20 nmol/mmp) were significant ($P < 0.05$).

With succinate as the substrate (Table II) AFB_1 at the three concentrations, inhibited the state 3 respiration by 3–35%. Scopoletin at 5–10 nmol/mmp concentrations stimulated (18% and 9% respectively) the succinate dependent state 3 respiration but inhibited it at the higher concentration of 20 nmol/mmp, by about 9%.

Reduced cytochrome *c* dependent state 3 respiration (Table III) was not significantly affected by both scopoletin and AFB_1 at 5 nmol/mmp concentration. However, at higher concentrations (10–20 nmol/mmp), AFB_1 and scopoletin (at 20 nmol/mmp only) inhibited the respiration by about 21 and 36% respectively.

Effects of scopoletin and AFB_1 on Respiratory control

The effects of scopoletin and AFB_1 on the bovine liver mitochondrial respiratory control ratio (RCR) shown in Tables I–III, indicate that the efects of the two compounds on the ratio was dependent on the respiratory substrate employed. With NAD^+ -linked isocitrate, it was observed that both scopoletin and AFB_1 caused marked increase (22–59%) in the RCR at the concentration range of 5–20 nmol/mmp ($P < 0.05$).

When the FAD^+ – linked succinate and the reduced cytochrome *c* dependent respiration were considered, it was observed that at 10–20 nmol/mmp concentrations, both scopoletin and AFB_1 significantly ($P < 0.05$) reduced the RCR by 22–39% in the case of succinate dependent respiration, and by 14–26% in the case of reduced cytochrome c dependent respiration. At 5 nmol/mmp, the two compounds did not elicit any observable change on the reduced cytochrome *c* dependent RCR.

Table III. Effects of scopoletin and aflatoxin B_1 on oxygen uptake of bovine hepatic mitochondrial reduced cytochrome c_1 dependent respiration.

* Concentration of compound (per mg mitochondrial protein)	State 4	State 3	Respiratory control ratio (RCR)	State 4 plus 2.4 DNP
Control (10% DMF)	12.48 ± 0.25	43.68 ± 0.85	3.50	13.28 ± 0.50
5 nmol AFB_1	12.58 ± 0.35	43.70 ± 0.87	3.47	13.00 ± 0.31
10 nmol AFB_1	12.68 ± 0.40	+#34.32 ± 0.91	2.71	13.18 ± 0.65
20 nmol AFB_1	12.45 ± 0.28	#34.30 ± 0.82	2.76	13.25 ± 0.02
5 nmol Scopoletin	@#21.84 ± 0.33	43.60 ± 0.92	2.00	#28.14 ± 0.31
10 nmol Scopoletin	@#15.16 ± 0.25	40.56 ± 0.54	2.60	#20.56 ± 0.98
20 nmol Scopoletin	#9.36 ± 0.91	#28.08 ± 1.02	3.00	#15.16 ± 0.42

States 4 and 3. Oxygen uptake were measured as nmol O_2/min x mg mitochondrial protein as indicated in Table I legend. Values presented are also as indicated in the legends. Tables I and II. All table signs are as indicated in Table I.

Effects of scopoletin and AFB_1 on state 4 Respiration in the presence of 2,4 DNP

The amount of oxygen uptake by the mitochondria was determined in the presence of the uncoupler, 2, 4 DNP. This was done in the presence of various concentrations of either AFB_1 or scopoletin as well as control, so as to ascertain the effects of both AFB_1 and scopoletin on oxygen uptake in the presence of the uncoupler (Obidoa and Siddiqui, 1978).

As shown in Tables I – III a comparison of the state 4 and state 4 plus 2, 4 DNP values, shows that the uncoupler 2, 4 DNP, did not relieve the AFB_1 – induced inhibition of the isocitrate NAD^+ dependent state 4 respiration. Both the succinate and reduced cytochrome *c* dependent respirations were also, not significantly ($P > 0.05$) altered by the various concentrations of AFB_1 in the presence of the uncoupler. The effects of scopoletin on the various respiratory states induced by the three substrates were generally enhanced in the presence of 2, 4 DNP.

Effects of scopoletin and AFB_1 on Ca^{2+} – induced swelling

Ca^{2+} -induced mitochondrial swelling is usually associated with the uptake of the cation by mitochondria (Lehninger *et al.*, 1967). Both AFB_1 and scopoletin elicited significant ($P < 0.05$) increases in the Ca^{2+} – induced mitochondrial swelling relative to control (Table IV). The swelling was in each case, reversed by ATP (data not presented) indicating that the mitochondria were intact, and that the reversible osmotic response of the mitochondrial membrane were unaltered (Obidoa and Ngodo, 1984). The scopoletin – induced increases in the swelling were concentration dependent, while those due to AFB_1 were inversely related to the concentration of the compound. The increases elicited by scopoletin were generally higher ($P < 0.05$) than those elicited by AFB_1.

Discussion

The result of these experiments suggest that in bovine (*Bos indicus*) liver mitochondria, AFB_1 may be blocking electron transfer from NADH to coenzyme Q (complex 1) since electron transfer (respiration) within this complex was generally inhibited (Table I) by it. On the other hand, electron transfer (respiration) from succinate through FAD to coenzyme Q (complex II), and from reduced cytochrome *c* to cytochrome oxidase (complex IV), remained either stimulated or unaffected by the compound (AFB_1) as shown in Tables II-III. With scopoletin on the other hand, electron transfer from complex I to complex III was generally stimulated (Tables I and II). These results indicate that while AFB_1 inhibited the electron transport at complex I, scopoletin stimulated it. The stimulation of electron transport (respiration) in the presence of 2,4 DNP (Tables I-III), and enhancement of Ca^{2+} induced swelling (Table IV) by scopoletin, as well as the inhibition of electron transport (respiration) in the presence of 2,4 DNP by AFB_1, with only marginal enhancement of Ca^{2+} – induced (Table IV) by AFB_1, further buttress the possible uncoupling effects of scopoletin and the inhibitory effects of AFB_1 on bovine liver mitochondrial respiration.

Scopoletin and AFB_1 increased the RCR for isocitrate respiring mitochondria (state 3) indicating more tightly coupled mitochondria. The value

Table IV. Effects of scopoletin and aflatoxin B_1 on Ca^{2+} -induced swelling of bovine hepatic mitochondria.

Conc. of compound (per mg Mitochondrial Protein)	Control 10% DMF	5 nmol AFB_1	10 nmol AFB_1	20 nmol AFB_1	5 nmol Scopoletin	10 nmol Scopoletin	20 nmol Scopoletin
% Ca^{2+}-induced swelling	7.57 ± 0.50	#12.63 ± 1.07	#12.07 ± 0.67	#10.87 ± 0.67	@#26.70 ± 0.93	@#33.50 ± 1.21	@#35.20 ± 0.08

Percentage change in Ca^{2+} -induced mitochondrial swelling were calculated from the difference between the absorbance of mitochondrial suspension at 520 nm at zero time and after 8 minutes for both the control and test experiments as described in Methods. Values presented are mean ± S. D. of four replicate data pooled from two different mitochondrial preparations. The suspension medium used for the experiments was described in the text. All table signs are as described in Table I.

of the RCR decreased when succinate and reduced cytochrome *c* were the respiratory substrates, indicating that the mitochondria were uncoupled. In contrast with these observations, AFB_1 had earlier been reported to inhibit electron transfer between cytochrome *b* and c_1 (complex III) (Pai *et al.*, 1975; Doherty and Campbell, 1973) in rat liver mitochondria; and complexes I and II in guinea fowl liver mitochondria (Obidoa and Siddiqui, 1978) at equivent concentrations with those used in this experiment. It has also been observed that AFB_1 uncoupled rat liver mitochondria (Pai *et al.*, 1975; Bababunmi and Basir, 1972) although this uncoupling effect of AFB_1 was not observed in guinea fowl liver mitochondria (Obidoa and Siddiqui, 1978).

Doherty and Campbell (1973) had reported the inhibition by AFB_1 of the electron flow in actively respiring rat liver mitochondria following oral administration of the toxin, while Clifford and Rees (1967) observed no change in the phosphorylating activities under similar conditions as had been previously indicated by Svoboda *et al.* (1966). Pai *et al.* (1975) showed that AFB_1 could elicit both inhibitory and uncoupling effects on rat liver mitochondria, and that these effects were more pronounced at the respiratory site III. However, Obidoa and Obonna (1979, 1981) showed that the *in vitro* inhibitory effects of AFB_1 on rat liver mitochondria were manifested on both sites 1 and II. Although the present *in vitro* studies seem to agree with the inhibitory and stimulating effects of AFB_1, they seem to indicate that the inhibitory effects of AFB_1 were more directed towards respiratory site I than site II. These differences could be species – dependent as earlier suggested by Obidoa and Siddiqui (1978). The observed effects of 2, 4 DNP in the present investigation could also, be interpreted as further support for the proposal that the effects of AFB_1 were more on the membrane dependent electron transport than on the phosphorylation reactions (Obidoa and Obonna, 1981). This could also be applicable to the effects of scopoletin.

In general, this report indicates that at very low concentrations (nmol/mmp), AFB_1 showed higher tendency ($P < 0.05$) to inhibit the bovine liver mitochondrial respiration than scopoletin; while scopoletin showed higher tendency ($P < 0.05$) to uncouple the respiration than AFB_1. The respective effects of scopoletin and AFB_1 on mitochondrial respiratory complexes could be part of the structure – activity relationship of naturally occurring coumarin compounds, as it is already known that the hydroxycoumarins exert profound effects on mitochondrial functions (Pai *et al.*, 1975).

Although it might be difficult to assess the exact *in vivo* aspects of the scopoletin and AFB_1 interruption of the bovine mitochondrial respiration based on these *in vitro* studies, it could still be reasonable to predict the toxic potentials of the compounds to the bovine species along these lines. However, the extent of the AFB_1 -induced inhibition of the bovine hepatic mitochondrial respiration (3–35%) indicated in this study, is appreciably lower ($P < 0.05$) than the values (22–75%) reported for rat (Pai *et al.*, 1975; Doherty and Campbell, 1973) and the values of 80–100% reported for guinea fowl (Obidoa and Sidddiqui, 1978) for equivalent amounts of the toxin. This could be an indication that the toxicity of AFB_1 (and scopoletin) to the bovine species along these lines might be lower than to rats and guinea fowls. This could be related to the differential susceptibility of animal species to xenobiotics. In other words, rats and guinea fowls could be more responsive to the toxic effects of AFB_1 (and scopoletin) than cows.

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Bababunmi E. A. and Basir O. (1972). Effects of aflatoxin B_1 on the swelling and adenosine triphosphatase activities of mitochondria isolated from different tissues of the rat. FEBS Lett. **26**, 102–104.

Boyd J. N., Babish J. C. and Stoewsand A. S. (1982), Modification by beet and cabbage diets of AFB_1 induced rat plasma foetoprotein elevation, hepatic tumorigenesis and mutagenecity of urine. Food. Chem. Toxicol. **20**, 47–52.

Clifford J. I. and Rees K. R. (1967), The action of aflatoxin B_1 on the rat liver. Biochem. J. **102,** 65–75.

Detroy R. W., Lillehoj E. B. and Ciegler A. (1971), Microbiological Toxins, VI. Academic Press, New York, p. 3.

Doherty W. P. and Campbell T. C. (1973), Aflatoxin inhibition of rat liver mitochondria. Chem. Biol. Inter **7**, 63–77.

Eastbrook R. W. (1967), Mitochondrial respiratory control and the polarographic measurements of ADP: O ratios. In: Methods in Enzymology **10**, 41–45 (Eastabrook, R. W. and Pullman, M. E. eds) Academic Press Ltd. New York.

Heathcote J. C. and Hibbert J. R. (1978), Aflatoxins: Chemical and Biological Aspects. Elservier Scientific Publishing Co. Amsterdam, Oxford, New York, 16–53.

Hendrickse R. G., Lamplugh S. M. and Maegraith B. G. (1983), Aflatoxin and kwashiorkor: Epidemiology and clinical studies in Sudanese children and findings in autopsy liver samples from Nigeria and South Africa. Bull. Soc. Expt. Path. **76,** 559–566.

Johnson D. and Lardy H. (1967), Isolation of liver and kidney mitochondria. In: Methods in Enzymology. **10**, 94–96. (Eastabrook, R. W. and Pullman, M. E. eds). Academic Press Ltd. New York.

Lehninger A. L., Carafoli A. L. and Rossi C. S. (1967), Energy linked ion movement in mitochondrial systems. Adv. Enzymol. **29**, 259–267.

Minamikawa T., Akazawa T. and Uritani I. (1967), Analytical study of umbelliferone and scopoletin synthesis in sweet potato roots infected by *Ceratocystis fimbriata*. Plant physiol. **38**, 439–494.

Newberne P. M. and Butler W. H. (1969), Acute and chronic effects of aflatoxin B_1 on the liver of domestic and laboratory animals. A Review. Cancer Res. **29**, 236–250.

Obasi S. C. and Obidoa O. (1994), Effects of aflatoxin B_1 and scopoletin on bovine hepatic mitochondrial dehydrogenase *in vitro*. 1. α – Ketoglutarate and succinate dehydrogenses. *Nig. J. Biochem.* **9**, (In Press).

Obasi S. C., Njoku O. U. and Obidoa O. (1994), Effects of single oral doses of scopoletin and aflatoxin B_1 on the clotting time, serum cholesterol and phospholipid levels of chicks. Ind. J. Physiol. Pharmacol. **38**, 89–94.

Obasi S. C. and Obidoa O. (1995), Urinary excretion of scopoletin in humans following cassava (gari) diet consumption. West Afr. J. Biol. Scs. (WAJBS) **3** (1& 2), 94–98.

Obasi S. C., Njoku O. U., Obidoa O. and Ononogbu I. C. (1996), Effects of single oral dose of scopoletin and aflatoxin B_1 on the clotting time, serum cholesterol and phospholipid levels of guinea pigs. Nutr. Res. **16** (4), 667–672.

Obidoa O. and Siddiqui H. T. (1978), Aflatoxin inhibition of avian hepatic mitochondria. Biochem. Pharmacol **27**, 547–550.

Obidoa O. and Obonna E. (1979), DiHydroaflatoxin inhibition of energy – linked reduction of endogenous nicotinamide nucleotide in isolated rat liver mitochondria. Food Cosmet. Toxicol. **71**, 501–504.

Obidoa O and Obonna E. (1981), Aflatoxin inhibition of reversed electron transfer in rat liver mitochondria *in vitro*. Biochem. Med. **26**, 1–7.

Obidoa O. and Ngodo V. O. S. (1984), Effects of prolonged consumption of gari (cassava, *Manihot utilisima*) on rat hepatic energy metabolism. I. Mitochondrial respiratory control. Plant. Fds. Hum. Nutr. **34,** 159–168.

Obidoa O. and Obasi S. C. (1991), Coumarin compounds in cassava diets: 2 Health implications of scopoletin in gari. Plant. Fds. Hum. Nutr. **41**, 283–289.

Olorunsogo O., Uwaifo A. O. and Malomo S,O. (1990), Comparative effects of three naturally occurring furanocoumarins on mitochondrial bioenergetics. Chem. Biol. Interac. **74,** 263–274.

Pai M. R., Bai N. J. and Venkitasuberamanian T. A. (1975), Effects of aflatoxin on oxidative phosphorylation of rat liver. Chem. Biol. Interac. **10**, 123–131.

Svoboda D., Grady H. J. and Higgison J. (1966), Aflatoxin B_1 injury in rat and monkey liver. Am. J. Pathol. **49**, 1023–1051.

Thorne C. J. R. (1984), Techniques for determining protein concentration. In: Techniques in the Life Sciences. Vol. B1/1, B104/8. Elservier, North Holland.

Uzoho C. A. (1991), Comparative effects of scopoletin and aflatoxin B_1 on erythrocyte membrane ATPases of the goat and cow. MSc. Thesis. Biochemistry Department, University of Nigeria, Nsukka.

Werther J. L. (1980), Food and cancer. *New York State Journ. Med.* **80,** 1400–1420.

WHO (1979), Environmental Health Criteria. 11: Mycotoxins. WHO Publishers Geneva.