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EFFECTS OF SINGLE ORAL DOSES OF SCOPOLETIN AND AFLATOXIN B_1 ON THE CLOTTING TIME, SERUM CHOLESTEROL AND PHOSPHOLIPID LEVELS OF CHICKS

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Abstract : The plasma cholesterol and phospholipid levels as well as the bleeding time of chicks treated with single oral doses of scopoletin (60 μg/kg, body wt) and aflatoxin B_1 (50 μg/kg, body wt) were measured at intervals for a period of one week (168 h). Both compounds generally increased the bleeding time (AFB_1 0.8-28.7%, Scopletin 0.5-38.2%), serum total and free cholesterol, and the serum phospholipid levels but decreased the levels of the serum esterified cholesterol fraction relative to control throughout the period of study. The extent of these changes elicited by the respective compounds and the variation in the differences between their respective effects varied with the measured parameters. The importance of the similarities in the effects elicited by aflatoxin B_1 and Scopoletin was highlighted.

Key words : aflatoxin B_1 scopoletin chicks plasma cholesterol and phsopholipid levels increases

INTRODUCTION

Non-nutrient components of foods are known to play important roles in the aetiology of chronic and degenerative disease processes in experimental animals (1). Usually, changes in cholesterol and phospholipid levels could arise in response to alternations in metabolic processes. Some of these alternations could be indices for pathological manifestations in such disease conditions as cancer. They could also predispose to hyperlipidaemia which is a common feature of artherosclerosis the complications of which lead to ischaemic heart disease, myocardial infarction and stroke (2).

Processed cassava diets (particularly gari) have been implicated in a number of human disease states usually presenting gross manifestations (3-7) and most frequently chronic cytogenic lesions (8-11). They have also been shown to elicit lipid metabolic disorders in laboratory animals (12-16). These conditions have previously been attributed to the cumulative toxic manifestations of the sub-lethal doses of cyanide present in such diets following their prolonged consumption (5, 17-19). However, the highly insignificant levels of cyanide in processed cassava foods (20, 21) raises doubts as to the exclusive role of cyanide in cassava, in processed cassva diet (e. g gari)-induced toxicities. Consequently, the role of other non-nutrient constituents of cassava in processed cassava diet-induced toxicities have been suspected (10, 15).

In a recent study, Obidoa and Obasi (22) indicated

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the presence of significant levels (50-70 µmoles/100 g dry wt) of scopoletin (6 methoxy-7-hydroxy coumarin) in processed cassava diets like gari and cassava flour. The levels of the compound in such foods were unaltered by either processing or storage. Also, a pharmacokinetic study of the compound in human subjects showed a possible retention of about 15% of the dietary (gari) scopoletin in the body (23). Apart from scopoletin, aflatoxin B_1 (AFB_1) a known toxigenic bisfuranocoumarin compound has been shown to be present in improperly stored cassava food products (24,25) and possibly gari. AFB_1 could also be retained in humans exposed to diets contaminated by the compound (26). However, unlike AFB_1, there has been a dearth of information on the toxigenic potentials of scopoletin in man and other animal systems.

The present study is aimed at elucidating the comparative toxic potentials of scopoletin using AFB_1 as a positive reference toxic coumarin compound. This is with a view to determining the contributory roles of the compound (Scopoletin) in cassava diet (particularly gari)-induced toxicities especially those related to lipid metabolism. Therefore, we have investigated the effects of single sub-lethal oral doses of scopoletin and AFB_1 on the bleeding time, serum cholesterol and phospholipid levels in an avian model that is susceptible to aflatoxicoses (27).

METHODS

Animals and treatment: Sixty (two-week-old) Nera Cockrels (Hypeco-Holland) having a mean weight of 135±25 g and purchased from a local poultry farm were used for these experiments. The chicks were housed in raised steel cages in the animal house (28±2°C) of the Department of Biochemistry, University of Nigeria, Nsukka. The animals were separated into two test (A and B) and one control (C) groups of twenty animals each. Animals in group A were each given 60 µg/kg body weight doses of scopoletin (Serva Fein Biochemicals Heidelberg/New York) orally, while the group B animals were each given orally 50 µg/kg body weight of AFB_1 (Sigma Co. St Louis USA). The group C animals were also orally treated with the equivalent volume of aqueous (10%) N, N-dimethylformamide (Merck) solution which was the solvent for both scopoletin and AFB_1. After the treatments, all the animals were allowed free access to feed (Top feeds ; 72.5% Carbohydrate 26.5% crude protein, and 1.0% oil) and water *ad lib.*

Bleeding time: The bleeding time was determined by the time it took for ceasation of bleeding following a slight cut-off of the toe-tip of the chicks. Ceasation of bleeding was determined by filter-paper blot procedure as described by Brown (28).

Serum lipid level estimation : Sera were collected from the blood samples obtained from the chicks through cardiac puncture. Serum total free and esterified cholesterol levels were determined by the methods described by Searcy and Beraquist (29) while the phospholipid levels were by the method of Stewartt (30).

The various parameters - bleeding time and serum lipid level estimations, were in each case determined immediately after the respective treatments corresponding to the zero hour (0h) and at 6, 24, 48 and 168 hour time intervals after the respective treatments. At each of these periods, four animals were taken from the respective groups (test and control group) for the various determinations. Percentage change in the various parameters were calculated with respective mean values. Statistical analysis was by students 't' test.

RESULTS

Bleeding time : Both scopoletin and AFB_1 generally elicited increases (relative to the control animals) in the bleeding time of the chicks treated with the compounds. As indicated in Fig. 1, the percentage increases elicited by scopoletin treatment were generally higher (0.5-38%) than those due AFB_1 (0.8-29%) treatment. However, the difference in the percentage increases elicited by scopoletin and AFB_1 treatments were not statistically significant ($P > 0.05$).

Serum lipid levels: There were increases in the serum total and free cholesterol (Fig. 2) as well as the phospholipid levels (Fig.3) of the chicks treated with scopoletin and AFB_1 when compared with the control animals. However, both compounds elicited a general decrease in the esterified cholesterol levels in the treated relative to the control chicks (Fig. 2).

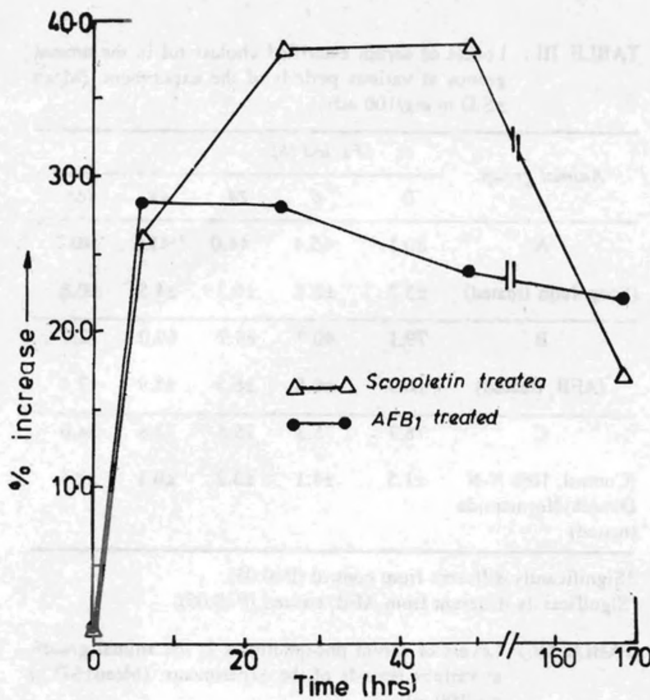

Fig. 1 : Time course of percentage change relative to control in the blood clotting time of chicks treated with scopoletin and AFB_1.

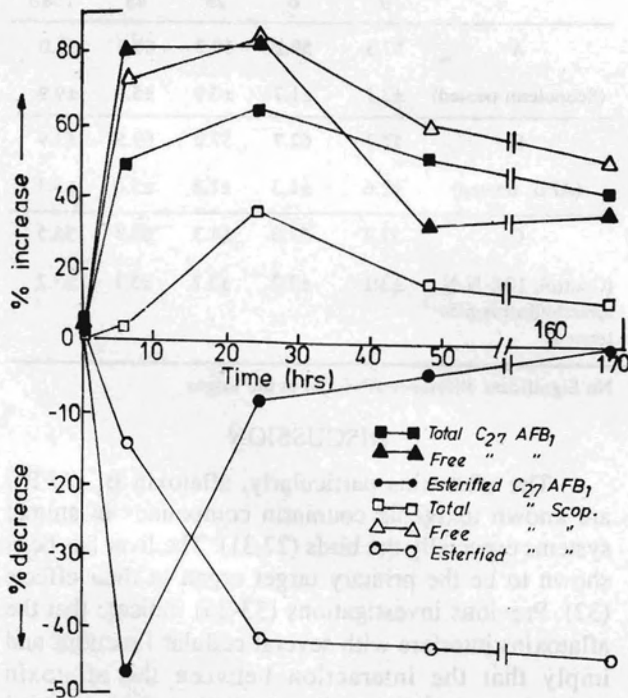

Fig. 2 : Time course of the percentage change relative to control in the serum total free and esterified cholesterol (C_{27}) levels of chicks treated with scopoletin and AFB_1.

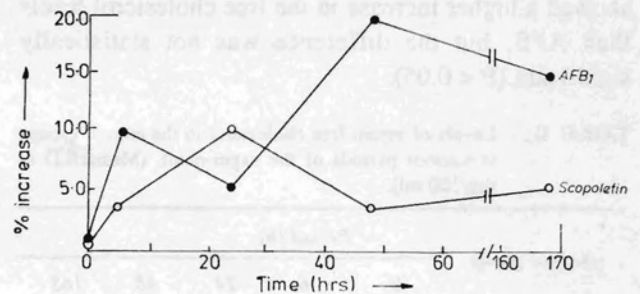

Fig. 3 : Time course of the percentage change relative to control in the serum phospholipid levels of chicks treated with scopoletin and AFB_1.

As shown in Table I, the increases (relative to control) in the serum cholesterol levels elicited by AFB_1 was significant ($P < 0.05$) from the 6th hour period while that due to scopoletin was significant ($P < 0.05$) from the 24th hour period. Also, the increases in the serum total cholesterol levels elicited by AFB_1 were significantly ($P < 0.05$) higher than those due to scopoletin.

TABLE I : Levels of total serum cholesterol in the animal groups at various periods of the experiment. (Mean ± SD in mg/100 ml).

Animal group	Period (h)				
	0	*6*	*⁺24*	*48*	*168*
A	137.6	⁺142.0	⁺179.7	⁺164.0	⁺149.9
(Scopoletin treated)	±3.6	±7.6	±5.8	±6.1	±4.1
B	144.7	*203.3	*218.6	*207.9	*191.2
(AFB_1 treated)	±6.1	±6.8	±5.7	±5.6	±3.6
C	135.2	135.5	132.1	138.2	134.1
(Control, 10% N, N Dimethylformamide treated).	±3.6	±3.5	±5.6	±6.8	±5.2

*Significantly different from control (P<0.05)

⁺Significantly different from AFB_1 treated (P<0.05)

Both scopoletin and AFB_1 treatments elicited significantly higher increases ($P < 0.05$) in the serum free cholesterol levels when compared with the control

animals (Table II). Table II also indicates that scopoletin showed a higher increase in the free cholesterol levels than AFB_1 but the difference was not statistically significant ($P < 0.05$).

TABLE II : Levels of serum free cholesterol in the animal groups at various periods of the experiment. (Mean±S.D in mg/100 ml).

Animal group	*Period (h)*				
	0	*6*	*24*	*48*	*168*
A	57.3	*103.8	*104.8	*90.7	*79.7
(Scopoletin treated)	±5.2	±3.6	±7.2	±3.2	±6.5
B	57.5	*106.5	*104.3	*74.5	*72.2
(AFB_1 treated)	±3.8	±9.1	±4.3	±3.1	±0.7
C	57.0	59.3	57.2	56.3	53.1
(Control, 10% N-N Dimethylformamide (treated)	±5.6	±3.6	±2.3	±3.2	±5.1

*Significantly different from control ((P<0.05)

The two compounds elicited decreases inthe serum esterified cholesterol levels in the treated animals when compared with the untreated (control) animals (Fig.2). While the decrease in the serum esterified cholesterol levels due to scopoletin was significant ($P < 0.05$) especially after the 6th hour, that due to AFB_1 was not significant after this period (Table III). Invariably, the effects of the AFB_1 treatment on the serum esterified cholesterol levels seem to be eliminated with time unlike those due to scopoletin (Fig. 2). Generally the decrease in the esterified cholesterol levels elicited by scopoletin was significantly ($P < 0.05$) higher than that due to AFB_1 (Table III).

The increases elicited by both scopoletin and AFB_1 on the serum phospholipid levels (Fig.3) of the treated animals relative to the control were not significant ($P > 0.05$) (Table IV). Although AFB_1 tended to show a higher increase in the phospolipid levels than scopoletin, the difference was also not statistically significant ($P > 0.05$).

TABLE III : Levels of serum esterified cholesterol in the animal groups at various periods of the experiment. (Mean ±S.D in mg/100 ml).

Animal group	*Period (h)*				
	0	*6*	*24*	*48*	*168*
A	80.3	+65.4	*+44.0	*+41.5	*+40.7
(Scopoletin treated)	±3.7	±3.8	±0.2	±4.5	±0.5
B	79.1	40.7	69.7	69.0	72.7
(AFB_1 treated)	±0.9	±6.8	±6.5	±8.9	±7.4
C	78.7	76.3	75.5	72.6	74.0
(Control, 10% N-N Dimethylformamide treated)	±1.5	±4.1	±3.2	±0.1	±2.5

*Significantly different from control (P<0.05).
+Significantly different from AFB_1 treated (P<0.05).

TABLE IV : Levels of serum phospholipids in the animal groups at various periods of the experiments. (Mean±SD in mg/100 ml)

Animal group	*Period (h)*				
	0	*6*	*24*	*48*	*168*
A	57.3	59.2	59.7	60.6	59.0
(Scopoletin treated)	±3.1	±1.7	±0.9	±5.7	±9.9
B	57.7	62.7	57.2	69.5	63.9
(AFB_1 treated)	±2.6	±1.3	±1.8	±5.0	±9.3
C	57.2	57.0	54.3	58.3	55.5
(Control, 10% N-N dimethylformamide treated)	±3.1	±7.2	±3.2	±5.1	±3.2

No Significant difference (P>0.05) in the values.

DISCUSSION

The aflatoxins particularly, aflatoxin B_1 (AFB_1) are known toxigenic coumarin compounds in animal systems especially the birds (27,31). The liver has been shown to be the primary target organ in their effects (32). Previous investigations (33-35) indicate that the aflatoxins interfere with several cellular functions and imply that the interaction between the aflatoxin molecules and the liver cells apparently occurs at several

loci. This leads to alterations in several metabolic systems in the animals susceptible to aflatoxicoses.

The present study has shown that single sub-lethal oral doses of both AFB_1 and scopoletin elicited a general alteration in the bleeding time, serum cholesterol and phospholipid levels in the chicks. Since both blood clotting factor synthesis and lipid metabolism are hepatic events (36, 37), these alterations imply that both scopoletin and AFB_1 elicited alterations in hepatic events of these animals at very low concentrations.

Although the effect of AFB_1 on bleeding time of chicks has been shown earlier (38), not much is known about its effects on the lipid metabolism of the animals. Similarly, the pharmacological effects of scopoletin in animal systems particularly the chicks have hitherto been unknown. Therefore, in addition to being in agreement with the findings that AFB_1 elicits appreciable increases in the bleeding time of the chicks (38), our present study also shows that the compound alters some lipid metabolic systems in such animals. This is the first report which shows that scopoletin increases the bleeding time and alters lipid metabolic patterns in the chicks. These effects showed identical patterns with those of the toxigenic compound, AFB_1. The two compounds differed only in terms of degree which may be attributed to their structural differences since both are coumarins. The similarities could have some obvious implications in the aetiology of certain disease conditions related to the disorders of lipid metabolism in the birds and possibly man. This is because aflatoxin contaminated diets as well as scopoletin containing foods such as cassava diets and potatoes (39, 40) are widely consumed by man. Further studies with multiple doses of these compounds and investigations in other animal models might be necessary to establish these ascertions.

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