Effect of the leafy vegetable *Solanum nigrum* on the activities of some liver drug-metabolizing enzymes after aflatoxin B_1 treatment in female rats

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Wistar albino female rats were maintained for 10 d on diets containing various levels of the vegetable *Solanum nigrum*. Simultaneously, they received daily intraperitoneal injections of aflatoxin B_1 (AFB_1) (either 0·2 or 0·4 mg/kg body-weight) diluted in propylene glycol. At the end of the experiment, all animals were killed and their serum and hepatic microsomes were prepared for assay of enzymes. Results showed that aminopyrine N-demethylase activity increased 2·5-fold with 200 (S_{200}) and 600 (S_{600}) g *S. nigrum*/kg diets. Activity of uridine diphosphate glucuronyltransferase (UDPGT) (*EC* 2.4.1.17) also increased twofold. Similar results were obtained with glutathione S-transferase (*EC* 2.5.1.18) activity which increased by 60% with diet S_{600}. After AFB_1 treatment, a general increase in the activities of the above enzymes was found, except for UDPGT in the group fed on diet S_{600}. When rats were fed on the diet without *S. nigrum*, AFB_1 induced an increase in alkaline phosphatase (ALP) (*EC* 3.1.3.1), aspartate aminotransferase (AST) (*EC* 2.6.1.1) and γ-glutamyltransferase (γ-GT) (*EC* 2.3.2.2) levels in the serum. AFB_1 also induced increases in serum ALP and γ-GT levels when rats were fed on diet S_{600}.

Aflatoxin B_1: Liver drug-metabolizing enzymes: Detoxification: Rat

Aflatoxin B_1 (AFB_1), a metabolite of the fungus *Aspergillus flavus*, is responsible for many toxic and carcinogenic effects observed in laboratory and domestic animals (Newberne & Butler, 1969; Newberne & Rogers, 1973). Several epidemiological studies indicate that AFB_1 intake is associated with a high incidence of primary liver cancer in man in Africa and Asia (Alpert *et al.* 1971; Peers *et al.* 1976; Sun & Chu, 1984). Many reports have shown that its content in some foods is more than tenfold the recommended maximum (Editorial, 1969; Nwokolo & Okonkwo, 1978; Domngang *et al.* 1984). In view of the fact that this metabolite can induce several toxic effects in the human body (Krishnamachari *et al.* 1975) it is important to find a method of detoxification, since people consume it daily in some areas. Nutrition is likely to be important in this respect.

Many workers have shown the importance of some nutrients, such as proteins and vitamins, in the metabolism and detoxification of AFB_1 (Campbell & Hayes, 1974; Domngang & Bassir, 1981; Parke & Ioannides, 1981). Thus, it has been shown that the susceptibility to toxic effects of AFB_1 varies greatly with nutritional status (Madhavan & Gopalan, 1965, 1968; Newberne & Rogers, 1973; Appleton & Campbell, 1983). Besides these effects, it is important to know what sort of diet, which is locally available, could help in detoxification of the toxin. Previous work in our laboratory has shown that some local foods containing the vegetables *Solanum nigrum* or *Amaranthus hybridus* increase the in vitro demethylation of AFB_1 and aflatoxin G_1, and the concentration of some drug-metabolizing enzymes (Domngang, 1985; Domngang *et al.* 1988).

* For reprints.

Table 1. *Experimental design*

Dietary group...	S_0	S_{200}	S_{600}
Treatment subgroup			
Control (A_0)	A_0S_0	A_0S_{200}	A_0S_{600}
AFB_1-treated groups (A_1)	A_1S_0	A_1S_{200}	A_1S_{600}
AFB_1-treated groups (A_2)	A_2S_0	A_2S_{200}	A_2S_{600}

AFB_1, aflatoxin B_1; A_0, 0·2 ml propylene glycol; A_1, 0·2 mg AFB_1/kg body-weight in 0·2 ml propylene glycol; A_2, 0·4 mg AFB_1/kg body-weight in 0·2 ml propylene glycol; S_0, S_{200} and S_{600} were diets containing 0, 200 and 600 g *Solanum nigrum*/kg.

The experiments reported in the present paper were designed to determine whether a local food containing *S. nigrum* could help AFB_1 detoxification in rats. Hepatic drug-metabolizing enzymes, including aminopyrine N-demethylase, uridine diphosphate glucuronyltransferase (*EC* 2.4.1.17; UDPGT), and glutathione S-transferase (*EC* 2.5.1.18) were analysed in order to elucidate this effect.

MATERIALS AND METHODS

Chemicals

Nicotinamide adenine dinucleotide phosphate (reduced form), propylene glycol, aflatoxin B_1, glutathione, *p*-nitrophenol and aminopyrine were brought from Sigma Chemical Co (St Louis, Mo, USA). Styrene oxide was purchased from Fluka AG (Basel, Switzerland). The other chemicals were of reagent grade.

Experimental diets

Leaves of *S. nigrum* (a grass from the family of Solanaceae) were bought from the local market every morning and cooked, while maize flour, palm oil and kitchen salt were mixed and cooked as described by Domngang *et al.* (1988). The cooked vegetable and cooked flour were then mixed in the following proportions to provide three different diets: 0:1000 (diet S_0), 200:800 (diet S_{200}), and 600:400 (diet S_{600}). These diets were then given to different groups of animals while sub-samples of diets were taken for analysis of nutrients.

Animals and treatment

Weanling female albino Wistar rats (Biochemistry Unit Animal House, University of Yaoundé) weighing 130–170 g, were divided into three dietary groups of eighteen animals each. They were housed individually in suspended wire-bottomed cages and fed on the appropriate experimental diet (S_0, S_{200} or S_{600}) and given tap water *ad lib.* for 5 d. After this acclimatization period each dietary group was divided into three subgroups as follows: a control (A_0) and two groups treated with 0·2 (A_1) or 0·4 (A_2) mg AFB_1/kg body-weight. AFB_1 was given to rats intraperitoneally in propylene glycol, while the control group received only the vehicle. Nine groups were thus arranged in a 3×3 (diet $\times$ AFB_1) factorial design (Table 1).

They were given the experimental diets every morning between 08.00 and 09.00 hours and injected with AFB_1 between 17.00 and 18.00 hours. Their weights were recorded every other day in order to assess weight gain or loss. The experimental treatment period was 10 d. After the last feeding day the rats were anaesthetized with diethylether and blood collected by cardiac puncture and serum prepared from it. Animals were then killed by decapitation and their livers removed quickly and washed in ice-cold 0·25 M-sucrose and 1 mM-Tris-

Table 2. *Nutrient composition of experimental diets given to rats*

(Mean values and standard deviation)

Diet...	S_0		S_{200}		S_{600}	
Constituents	Mean	SD	Mean	SD	Mean	SD
Lipids (g/kg DM)	117	14	105	8	102	17
Carbohydrate (g/kg DM)	742	—	734	—	584	—
Crude protein* (g/kg DM)	75	5	113	18	188	7
Fibre (g cellulose/kg DM)	47	3	30·4	1	104	4
Pectin	6·9	3	7·4	1·5	6·5	0·5
Minerals (mg/kg DM):						
Phosphorus	2500	310	2522	229	2316	199
Potassium	496	53	596	42	1623	9
Magnesium	1163	110	1309	152	2181	164
Iron	50	8	36	5	92	35
Copper	13	3	15	0	175	3
Zinc	27	5	32	2	39	2
Calcium	1150	43	1625	61	2095	42
β-Carotene (mg/kg fresh matter)	339	50	400	51	834	132
Vitamin C (mg/kg fresh matter)	Trace	—	66	7	229	12
Oxalic acid (g/kg DM)	3·2	0·09	3·6	0·4	5·6	0·3

DM, dry matter; S_0, S_{200} and S_{600} were diets containing 0, 200 and 600 g *Solanum nigrum*/kg respectively.
* Nitrogen × 6·25.

hydrochloric acid buffer (pH 7·4), blotted and weighed. Each liver was minced with scissors and homogenized in the same buffer. Microsomes and cytosol were isolated according to the procedure of Bock *et al.* (1983).

Assays

Protein was assayed by the method of Lowry *et al.* (1951) using bovine serum albumin as standard. Aminopyrine N-demethylase activity was assayed by the method of Aströn *et al.* (1983) and formaldehyde produced estimated by the procedure of Nash (1953). UDPGT activity was estimated as described by Bock *et al.* (1983) using *p*-nitrophenol as aglycone. Glutathione S-transferase activity was measured in the cytosol with styrene oxide as substrate (Baars *et al.* 1978).

The level of some hepatic lesion markers, aspartate aminotransferase (*EC* 2.6.1.1; AST), alkaline phosphatase (*EC* 3.1.3.1; ALP), γ-glutamyltransferase (*EC* 2.3.2.2; γ-GT) and β-glucuronidase (*EC* 3.2.1.31) were measured in the serum using assay kits (Sigma). The spectrophotometer used was a Beckman model 25.

Statistical analysis of the values was performed by analysis of variance (ANOVA) for a factorial experiment. Where the *F* test in ANOVA indicated a significant diet × AFB_1 interaction, the degree of significance of the difference between means for diets at a given AFB_1 dose for the liver microsomal enzymes, or the degree of significance of the difference between means for controls and respective AFB_1-treated rats for the serum enzymes, was determined by a *t* test.

RESULTS

Food composition of the three diets

The nutrient and amino acid composition of the experimental diets is shown in Tables 2 and 3 respectively. Addition of *S. nigrum* in diets S_{200} and S_{600} did not change the concentrations of lipids or phosphorus, but in diet S_{600} the concentration of carbohydrate

Table 3. *Amino acid composition (mg/g crude protein (nitrogen × 6·25)) of experimental diets compared with Food and Agriculture Organization/World Health Organization (FAO/WHO) standard combination (Watier, 1982)*

(Means of duplicate samples)

Amino acids	FAO/WHO standard	Diet		
		S_0	S_{200}	S_{600}
Isoleucine	40	51	48	36
Leucine	70	Traces	Traces	110
Lysine	55	40·4	195	471
Methionine + cysteine	35	50	105	141
Phenylalanine + tyrosine	60	84	76	61
Threonine	40	23	92	42
Tryptophan	10	7·1	14·8	44
Valine	50	Trace	Trace	6·1

There were important increases in the level of some essential amino acids (lysine, tryptophan, methionine + cysteine), whereas the amount of phenylalanine + tyrosine, and threonine decreased in diets S_{200} and S_{600}. Compared with FAO/WHO standard combination, diet S_0 was deficient in lysine, threonine, tryptophan, valine and leucine, diet S_{200} in valine and leucine while S_{600} was deficient in valine.

Diets S_0, S_{200}, S_{600} contained 0, 200 and 600 g *Solanum nigrum*/kg respectively.

was reduced, whereas that of fibre was increased. The concentrations of the following constituents were increased in diets S_{200} and S_{600}, with greater increases in S_{600}: protein, potassium, magnesium, copper, zinc, calcium, β-carotene, vitamin C and oxalic acid. The highest concentrations of essential amino acids were in diets S_{200} and S_{600} (Fig. 1 and Table 3).

Food consumption, body-weight and liver weight

Food consumption, body-weights and liver weights of the animals are shown in Table 4. Generally, the quantity of food consumed was low in AFB_1-treated groups. A loss of body-weight (2–9%) was observed in the AFB_1-treated groups and control group S_0. There were no significant differences in the relative liver weights of all groups.

Drug-metabolizing enzymes (Fig. 2)

Compared with the S_{200}- and S_{600}-fed groups, the aminopyrine N-demethylase activity was 150% lower in the control S_0-fed group. Treatment of rats with 0·2 mg AFB_1/kg body-weight increased the activity of this demethylase by 327 and 75% in groups S_0 and S_{200} respectively when compared with the respective controls. With 0·4 mg AFB_1/kg body-weight, the activity of this enzyme returned to its original level in the group fed on diet S_0, whereas there was a continued increase in groups fed on diet S_{200} (100%) and diet S_{600} (92%) (Fig. 2(*a*)).

As shown in Fig. 2(*b*) the activity of UDPGT was very low in the control group fed on diet S_0 when compared with those fed on diets S_{200} and S_{600}. But after administration of 0·2 mg AFB_1/kg body-weight, activity of this enzyme was increased by 111 and 99% in groups fed on diets S_0 and S_{200} respectively when compared with the group fed on diet S_{600}. On administration of 0·4 mg AFB_1/kg body-weight to rats, this enzyme activity displayed a similar pattern as described previously for 0·2 mg AFB_1/kg body-weight.

The activity of glutathione S-transferase (Fig. 2(*c*)) was higher in the S_{600}-fed group by 44 and 65% when compared with that of those fed on diets S_0 and S_{200} respectively. In the

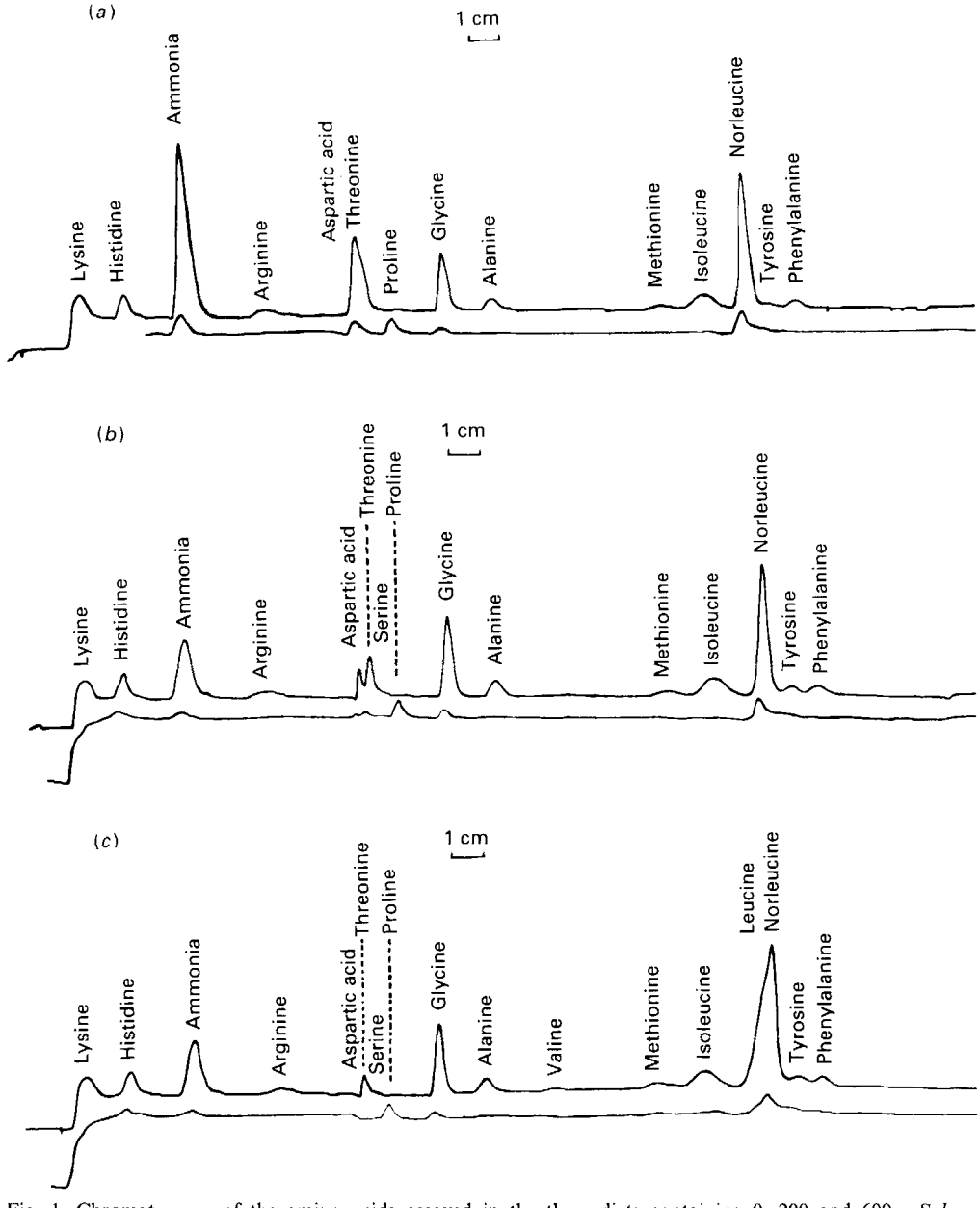

Fig. 1. Chromatogram of the amino acids assayed in the three diets containing 0, 200 and 600 g *Solanum nigrum*/kg (diets (*a*) S_0, (*b*) S_{200} and (*c*) S_{600} respectively). Samples (100 mg) were submitted to acid hydrolysis and analysed using a Technicon AutoAnalyser equipped with a chart recorder. The samples were run with an internal standard, norleucine, at a fixed concentration. There was a good separation of the basic amino acids; lysine, histidine and arginine, whereas aspartic acid, threonine and serine were not well separated as well as leucine and norleucine. Each amino acid was identified on the chart and the concentration calculated using the area of the peak Scale: 0·45.

Table 4. *The effects of aflatoxin B_1 (AFB_1; 0, 0·2 and 0·4 mg/kg body-weight intraperitoneally; A_0, A_1, A_2, respectively) treatment and dietary* Solanum nigrum *(0, 200 and 600 g/kg; S_0, S_{200} and S_{600} respectively) on average feed consumption (g/d), percentage weight gain or loss, and liver weight of female rats*

AFB_1 treatment...	Control group (A_0)			A_1			A_2		
Dietary *S. nigrum* level...	S_0	S_{200}	S_{600}	S_0	S_{200}	S_{600}	S_0	S_{200}	S_{600}
Average feed consumption	9·5	8·0	10·0	7·3*	5·7*	8·6	4·8***	6·0	8·2
Percentage weight gain or loss	−2·2	0·2	2·0	−5·0	−5·1	−6·1	−9·4	−5·9	−5·6
Liver (g/kg body-weight)	30	34	35	33	38	35	34	39	36

Mean values were significantly different from respective control group (SED 0·94): * $P < 0·05$, *** $P < 0·001$.

AFB_1-treated groups, the activity of this enzyme increased in group S_0 by about 50% and by 100% in group S_{200}, when compared with their respective controls. The higher dose of AFB_1 (0·4 mg/kg body-weight) caused the same changes as those described for the lower dose. The activity of glutathione S-transferase was not affected by AFB_1 in the group fed on diet S_{600}.

Enzyme levels in serum

The serum levels of the enzymes studied were increased following treatment of rats with AFB_1 in groups fed on diets S_0 and S_{600}. The increases were about 50% with ALP (Fig. 3(*a*)), 200% with γ-GT (Fig. 3(*d*)) and 47% with AST (Fig. 3(*b*)) (only with 0·4 mg AFB_1/kg body-weight) in the group fed on diet S_0 compared with the control group. In the S_{600}-fed group, the increases were of the same magnitude as for group S_0, except for γ-GT level which was slightly higher. However, the β-glucuronidase (Fig. 3(*c*)) activity did not vary significantly with diet or with AFB_1 treatment.

DISCUSSION

Maize flour and *S. nigrum* are among the most available and commonly consumed foods in rural areas in African countries. In designing our experiment, this type of food was taken into consideration. *S. nigrum* was used at different levels in order to improve the quality and quantity of different nutrients in the diets (Tables 2 and 3). AFB_1 was given intraperitoneally in order to separate materials, such as fibre, which could influence the absorption of the toxin, when this is given orally, as reported elsewhere (Shiau & Chang, 1986).

In the present study, the diet given to the rats led to a decrease in body-weight, in groups fed on diet S_0, when compared with the first day of experiment. Increasing the quantity of *S. nigrum* in the diets led to increasing weight gain. In terms of protein, diet S_0 was deficient, S_{200} was low, and S_{600} had a normal protein level. The concentrations of essential amino acids and other nutrients were increased by improving the amount of this vegetable in the diet; therefore, weight gain was observed in the group fed on diet S_{600}. AFB_1 induced weight loss in all dietary groups. This toxin is known to be distributed throughout the body after administration (Wogan *et al.* 1967) and one of its important actions is inhibition of protein synthesis (Sarasin & Moule, 1973).

In the control groups, the activities of all drug-metabolizing enzymes studied were raised in those fed on the vegetable diet. The addition of *S. nigrum* to diets (Table 2) improved the quantity of some nutrients having a direct influence on drug-metabolizing enzyme activities (protein, magnesium, vitamin C, β-carotene). These nutrients have been shown to

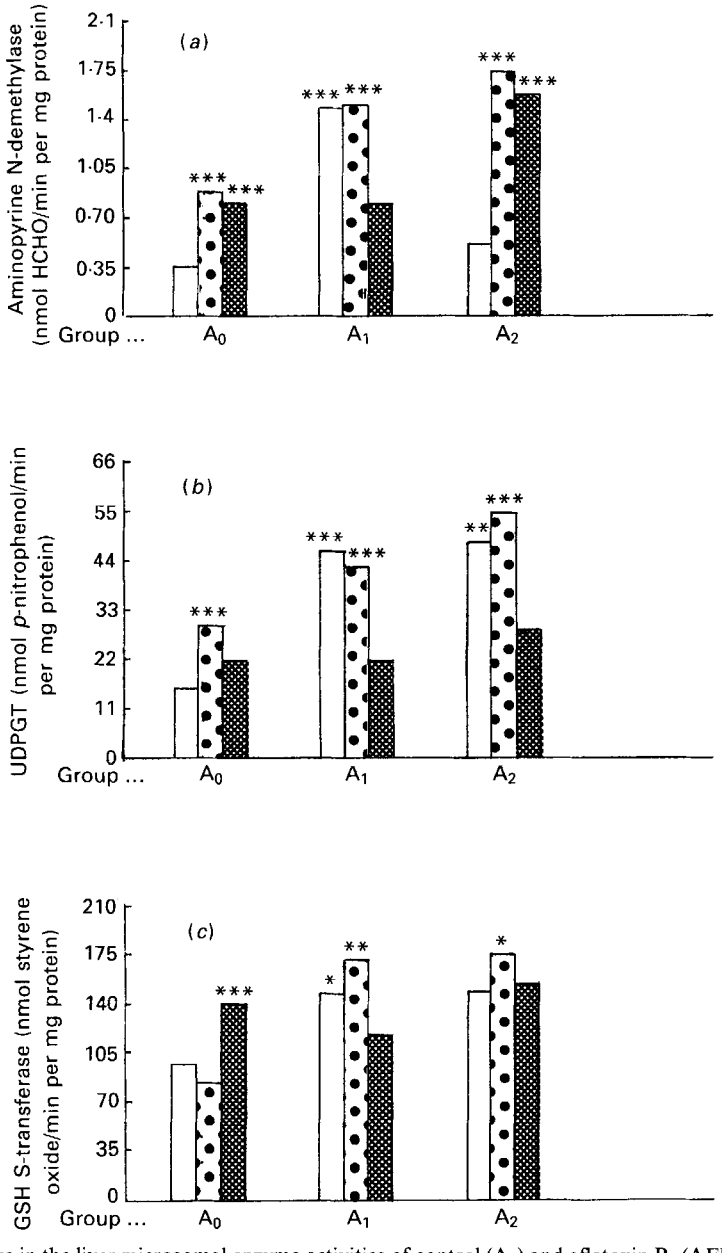

Fig. 2. Changes in the liver microsomal enzyme activities of control (A_0) and aflatoxin B_1 (AFB_1)-treated rats (0·2 and 0·4 mg/kg body-weight; A1 and A2 respectively) fed on diets containing 0 (S_0; □), 200 (S_{200}; ▨) and 600 (S_{600} ▩) g *Solanum nigrum*/kg. There was a significant difference between AFB_1 dose, but the difference was affected by the *S. nigrum* level in the diet, for (*a*) aminopyrine N-demethylase (AFB_1:diet effect $F = 70·03$, *f* diet/*f* $AFB_1 = 89·39/121·84$, $P < 0·001$; ANOVA), (*b*) uridine diphosphate glucuronyltransferase (*EC* 2.4.1.17; UDPGT) (AFB_1:diet effect $F = 8·39$, *f* diet/*f* $AFB_1 = 30·30/40·90$, $P < 0·001$; ANOVA), (*c*) glutathione (GSH) S-transferase (*EC* 2.5.1.18) (AFB_1:diet effect $F = 14$, *f* diet/*f* $AFB_1 = 2·85/37·84$, $P < 0·001$; ANOVA). Mean values for AFB_1 doses were significantly different: * $P < 0·05$, ** $P < 0·01$, *** $P < 0·001$.

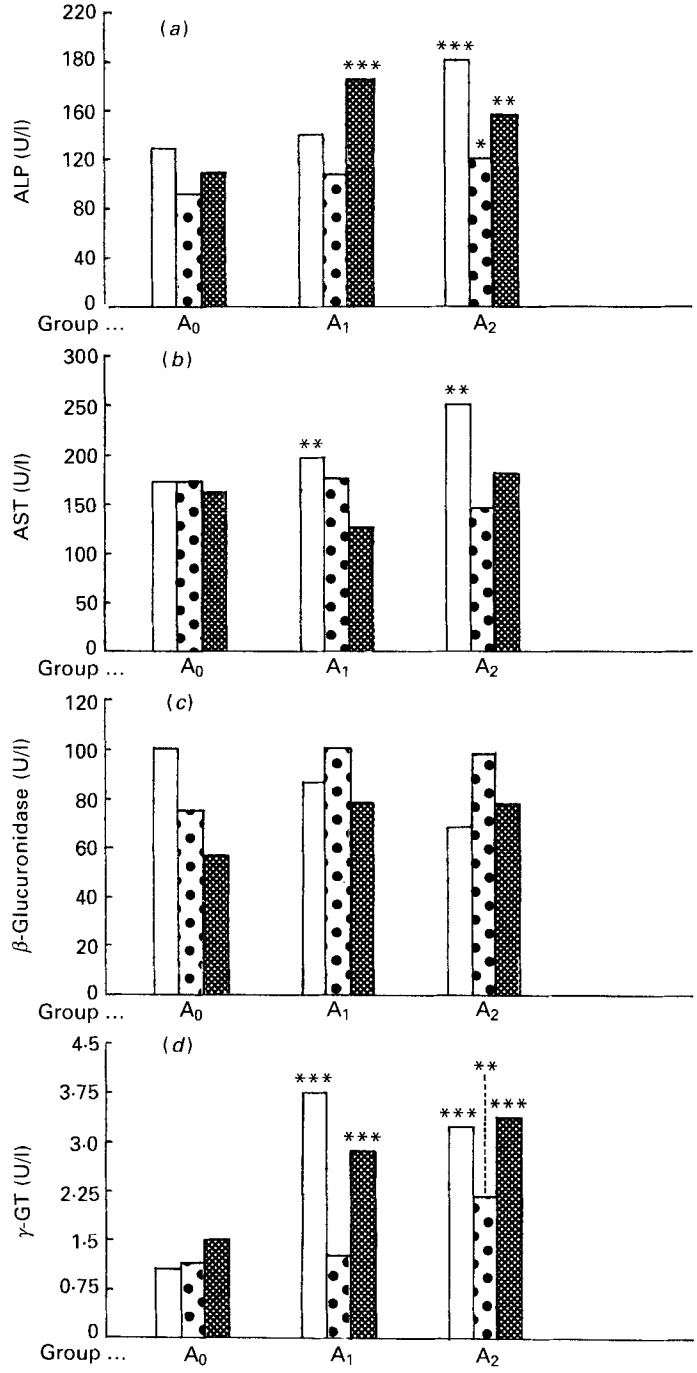

Fig. 3. Changes in the serum enzyme activities of control (A_0) and aflatoxin B_1 (AFB_1)-treated rats (0·2 and 0·4 mg/kg body-weight; A1 and A2 respectively) fed on diets containing 0 (S_0; □), 200 (S_{200}; ▣) and 600 (S_{600}; ▩) g *Solanum nigrum*/kg. There was a significant difference between AFB_1 doses, but the difference was affected by the level of *S. nigrum* in the diets, for (*a*) alkaline phosphatase (*EC* 3.1.3.1; ALP) (AFB_1:diet effect $F = 8{\cdot}51$, f diet/f $AFB_1 = 31{\cdot}69/27{\cdot}66$, $P < 0{\cdot}001$; ANOVA), (*b*) aspartate aminotransferase (*EC* 2.6.1.1; AST) (AFB_1:diet effect $F = 22{\cdot}35$, f diet/f $AFB_1 = 47{\cdot}33/14{\cdot}35$, $P < 0{\cdot}001$; ANOVA), (*d*) γ-glutamyltransferase (*EC* 2.3.2.2; γ-GT) (AFB_1:diet effect $F = 10{\cdot}75$, f diet/f $AFB_1 = 32{\cdot}98/65{\cdot}55$, $P < 0{\cdot}001$; ANOVA) but not for (*c*) β-glucuronidase (*EC* 3.2.1.31). Mean values for treated rats were significantly different from those for control rats: * $P < 0{\cdot}05$, ** $P < 0{\cdot}01$, *** $P < 0{\cdot}001$. U/l is the amount of enzyme (μg) which transforms 1 μmol/l per min.

induce the activity of these hepatic enzymes (Campbell & Hayes, 1974; Parke & Ioannides, 1981). Aminopyrine N-demethylase and UDPGT activities were high in both *S. nigrum*-fed groups, but were higher in the S_{200}-fed group than in the S_{600}-fed group. In contrast, glutathione S-transferase activity increased in the S_{600}-fed group. This could mean that the vegetable diet could induce better conjugation of AFB_1 derivatives.

Administration of AFB_1 led generally to an increase in the activity of the drug-metabolizing enzymes studied, regardless of dietary group. In addition, it lowered the feed intake per rat.

Dickerson *et al.* (1976) have shown that the activity of hepatic UDPGT was depressed in male rats given a low-protein, energy-restricted diet, and cytochrome P-450 was depressed by a low-protein *ad lib.*-energy diet. Hashmi *et al.* (1986) have shown that in female rats, food restriction by 25% did not alter the hepatic electron transport component, while restricting food by 50% lowered the level of the electron transport component and the glycogen content of the liver. Our results show that in the group with low food consumption (group A_2 S_0) (Table 4), microsomal aminopyrine N-demethylase activity was reduced. We also found that in the control S_0-fed group, the levels of drug-metabolizing enzymes were low.

In S_{200}- and S_{600}-fed groups, aminopyrine N-demethylase activities were raised after 0·4 mg AFB_1/kg body-weight treatment, while that of UDPGT increased only in the S_0- and S_{200}-fed groups. This increasing activity of UDPGT in the S_{200}-fed group may contribute to more excretion of hydroxylated AFB_1 derivatives. This may have a protective role as shown by Kamdem *et al.* (1982). The unchanged activity of UDPGT after AFB_1 treatment in the S_{600}-fed group may suggest a reduced ability of rats in this group to conjugate hydroxylated AFB_1 derivatives via the glucuronide route. Among these AFB_1 derivatives are aflatoxins M_1, Q_1 and R_0, which are considered to be as toxic as the parent compound (Campbell & Hayes, 1976). These toxins could be responsible for the toxicity observed in groups fed on diets S_0 and S_{600}, as shown by the increase in serum enzymes (AST, ALP and γ-GT). These enzymes are known to be good indicators of acute AFB_1 toxicity (Kamdem *et al.* 1981*a*,*b*, 1982). Another metabolite of AFB_1 which could be responsible for the toxicity observed in groups S_0 and S_{600} is AFB_1-dihydrodiol. This is derived from the epoxide through the intervention of epoxide hydratase, an enzyme whose activity has been shown to increase in female rats after chronic administration of AFB_1 (Kamdem *et al.* 1981*b*). This metabolite has a probable role in the toxicity of AFB_1, since its relative production by microsomes from various species parallels their in vivo susceptibilities to AFB_1 poisoning (Neal *et al.* 1981). Although epoxide hydratase yields this toxic metabolite, it also prevents the formation of AFB_1-DNA adducts, which could induce mutation. However, AFB_1-dihydrodiol could be rapidly conjugated by UDPGT and glutathione S-transferase, which increased in the group fed on *S. nigrum*. Conjugation to glucuronic acid and glutathione via UDPGT and glutathione S-transferase respectively, has been established as an important pathway in the detoxification and excretion of toxic metabolites of AFB_1 (Degen & Neumann, 1978).

The increase in serum enzyme activity (ALP, AST and γ-GT) after AFB_1 poisoning in groups S_0 and S_{600}, as shown by our results, is in good agreement with previous similar studies (Domngang *et al.* 1988). On the other hand, the constant level of AST and ALP in the S_{200}-fed groups after AFB_1 treatment suggests the possible importance of this diet in inducing excretion of the toxin. However, increasing the AFB_1 dose (0·4 mg/kg body-weight) induced increased serum γ-GT levels.

In conclusion, it appears that adding *S. nigrum* in a certain proportion to the local diet improves the quality and quantity of some nutrients, for example, vitamin C, β-carotene, protein and amino acids, which probably favour an increase in the activity of liver drug-

metabolizing enzymes. This food could help in the detoxification of AFB_1 which, according to many reports (Alpert *et al.* 1971; Nwokolo & Okonkwo, 1978; Domngang *et al.* 1988) is widespread in commonly consumed foods in Africa. Finally, the present study confirms that transaminases, ALP and γ-GT, could be used to monitor aflatoxin poisoning, as suggested previously (Kamdem, 1982).

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