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Full Length Research paper

Biochemical and histopathological changes in liver of albino rats fed diets incorporated with *Vernonia amygdalina* and *Vernonia colorata* leaves

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***Vernonia amygdalina* and *Vernonia colorata* are widely used in medicinal plant preparations in sub-saharan Africa. Certain dietary constituents can potentiate or reduce the potency of toxins such as AFB_1. The effect of dietary incorporation of *V. amygdalina* and *V. colorata* on some biochemical and histopathological indices in albino rats was studied. *V. amygdalina* and *V. colorata* were incorporated into diets compounded to isocaloric and isonitrogenous and fed to mature male alimo rats administered 250 mg/kg Aflatoxin B_1 over a 12 week period. Bioactivity studies using brine shrimp lethality test indicate that methanolic extracts of *V. amygdalina*. del and *V. colorata* had higher bioactivity (ED_{50} 121. 47 g/ml and 761.8 g/ml respectively) than their aqueous extracts 819.5 and 1,999.85 g/ml respectively). Alanine amino transaminase (ALT) activity increased significantly (P 0.05) relative to control group (Gp1) in groups fed *V. colorata* with or without AFB_1 treatment but showed no significant increase in groups fed *V. amygdalina* with or without AFB_1. Treatment of *V. colorata* fed animals with AFB_1 (Gp3) resulted in marked increase in alkaline phosphatase activity. Increase in Aspartate amino transaminase (AST) activities were not significantly different from those of animals fed only with *V. colorata* without AFB_1 . Administration of AFB_1 resulted in a significant increase in alkaline phosphatase activity in groups fed *V. colorata,* but not in groups fed *V. amygdalina*. Groups fed *V. amygdalina* + AFB_1 showed no significant changes in ALT and AST. These findings suggest that while the feeding of *V. amygdalina* may have hepatoprotective effects, the feeding of *V. colorata* may potentiate the toxic effects of toxins such as AFB_1. Histopathological studies on the liver show that the feeding of the two vegetable had varieties affect by the liver in different ways.**

Key words: *Vernonia amygdalin, Vernonia colorata,* AFB_1, serum enzymes, bioactivity.

INTRODUCTION

Vernonia amygdalina is widely used in the preparation of soups and porridges in south Eastern Nigeria. Two major varieties are used domestically. These two vary in their degree of bitterness (*Vernonia colorata* which is less bitter) and (*V. amygdalina.* del which is very bitter). *V. amygdalina* del is usually macerated extensively before use in soups and porridges but often used in unprocessed form in medicinal preparation. Its extract is used as a digestive tonic, appetizer, febrifuge and as antidiabetic tonic (Singha, 1966).

V. amygdalina var, is used in unprocessed forms as snack or in porridges. A number of the above uses have been investigated viz; antidiabetic activity (Akah and Okafor, 1992), antimalarial (Philipson et al., 1993) antiparastic activity (Ohigashi, 1995) and antimicrobial activity against organisms commonly incriminated in wounds (Ijeh et al., 1996).

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Table 1. Composition of diets for different groups of rats (g%).

Ingredient/feedstuff	Diet 1	Diet 11	Diet 111
Maize (flour)	50.50	50.50	50.50
Crayfish	4.20	3.36	3.36
Groundnut (meal)	9.29	7.43	7.43
V. *colorata*	-	4.61	-
V. amygdalina. del	-	-	4.33
Bone meal	4.00	4.00	4.00
Pre-mix	0.50	0.50	0.50
Oil	5.00	5.00	5.00
Corn-flour	26.50	24.50	24.88
Total (approx)	100.00	100.00	100.00

Table 2. Brine shrimp Lethality tests.

Extract	ED (50 glml)
Aqueous extract *V.a* var	1,999.85
Methanolic extract *V.a* var	761.89
Aqueous extract *V.a* del	819.59
Methanolic extract *V.a* del	121.47

Terpenoid extracts from *V. amygdalina* have been demonstrated to have hepatoprotective effects against Carbon tetrachloride induced liver damage in rats. Arhoghro et al. (2009) also reported that aqueous extracts of *V. amygdalina* del had hepatoprotective effects against Carbon tetrachloride induces liver damage in albino rats.

Liver diseases especially liver cancer is major cause of mortality and morbidity in the tropics. Hepatocellular carcinoma is the third leading cause of cancer deaths world wide with prevalence 16 to 32 times higher in developing countries where storage facilities are poor and risk of aspergillus flavus infestation of stored food products relatively higher than developed countries and temperate regions of the world (Liu and Wu, 2010). Numerous epidemiological studies have observed a correlation between ares of high aflatoxin B_1 exposure and high incidence of hepatocellulr carcinoma (Jackson and Groopman, 1999).

The feeding of AFB_1 along side *V. amygdalina* in this study is borne out of the need to investigate its possible effects on AFB_1 toxicity since research evidence has shown that certain dietary constituents can potentiate or reduce the potency of toxins such as AFB_1. AFB_1 is a major cause of hepatocarcinogenicity in Africa and Asia (Liu and Wu, 2010) . Antioxidant activities of flavonoids from *V. amygdalina* del have been previously reported (Igile et al., 1994). The two varieties of Vernonia most commonly consumed in the South east of Nigeria are often confused by inexperienced users. Several steroidal saponins and proteins found in *V. amygdalina* have been shown to have anticancer effect (Gresham et al., 2008).

MATERIALS AND METHODS

Plant materials

Stem cuttings of leaves of *V. amygdalina* and *V. colorata* were obtained from Okigwe Imp State, Nigeria and were botanically identified at the Botany Department of the University of Nigeria Nsukka. They were dried in mild sun for three days and then milled into fine particles using a Moulinex blender. They were stored in water proof bags in a deep freezer until ready for use.

Acute toxicity studies

Brine shrimp lethality tests were carried out using aqueous and methanolic extracts of the leaves of *V. amygdalina.* del and *V. colorata* as described by Maclaughin et al. (1991).

Experimental design and treatment of animals

Thirty male rats aged five to six weeks weighing 40 to 50 g were randomly assigned to six groups of five animals each. The animals were exposed to 12 h light and dark cycles under humid tropical conditions in stainless steel cages. Feed and water were supplied *ad libitum.*

After 7 days habituation on the basal diet, the animals were assigned randomly to separate cages and introduced to basal diet and diets compounded with the two different varieties under study. The composition of the diets is shown on Table 1. Diets were compounded to be isocaloric and isonitrogenous. Nutrients were supplied in amounts adequate for normal growth. Locally sourced ingredients were used. 250 g/kg body weight AFB_1 was administered *per os* by intubation for ten days to groups, 2, 4, and 6 commencing on the 10^{th} day of feeding the animals (post-equilibration). After AFB_1 dosing all the animals were fed for another nine weeks to complete 12 weeks of feeding post-equilibration. Animals were sacrificed by anaesthetizing with diethyl ether and venous blood was drawn by cardiac puncture and organs were blotted on filter paper and weighed. They were fixed in formal saline for histopathological studies.

Enzyme assays

Assays of Alanine amino tranaminase activity (ALT, EC, 2,6,1,2) and Aspartate amino transaminase activity (AST, EC. 2.6.1.1) were carried out as described of King (1978). Assay of Alkaline phosphatase activity was done as described by Teitz (1987).

Statistical analysis

Data obtained were analyzed using SPSS. The analysis of variance procedure for completely randomized design was used. Treatment means were separated. Multiple range tests were carried out using Scheffe's tests to separate statistically significant means at a significance level of 0.05

RESULTS

Table 2 shows result of bioactivity test. Methanolic were observed as shown on Plates 1 to 4. Alkaline extracts of

Plate 1. Photomicrograph of liver sections of rats fed the basal diet showing normal lobular architecture [H and E stain X 180] in rats fed basal diet.

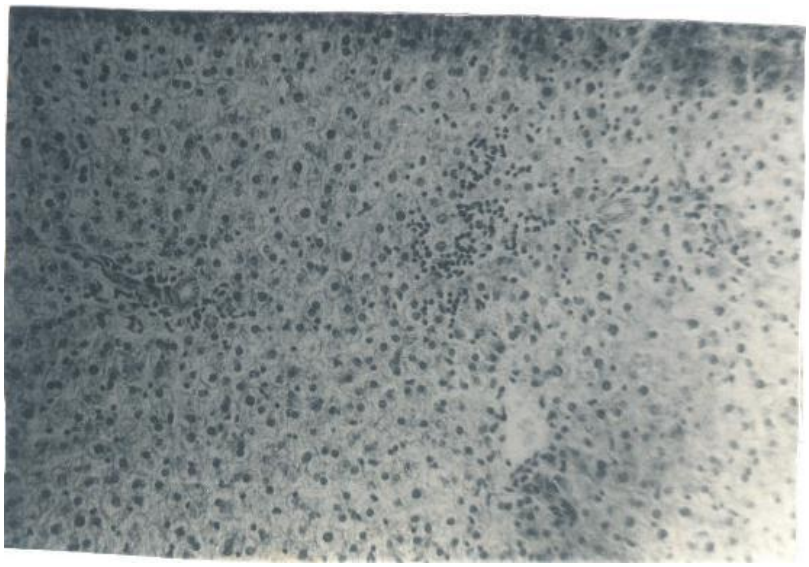

Plate 2. Photomicrograph of liver sections showing hepatocyte degeneration and necrosis with mononuclear cells infiltration of periportal areas [H and E stain X 180] in rats fed Basal diet +AFB_1.

V. amygdalina del showed the highest bioactivity (ED_{50} 121.47 g/ml) while aqueous extracts of *Vernonia colorata* showed the lowest bioactivity (ED_{50} 1, 999.8 g/ml). Remarkable changes in liver pathology phosphatase activity (Table 3) increased significantly in all groups treated with AFB $_1$ and also in the group fed *V. amygdalina* del only. ALT activity (Table 3) increased significantly in group fed *V. colorata* but not *V. amygdalina* del.

There was a non- significant ($p < 0.05$) increase in AST activity (Table 4) in all the vegetable diet groups with or without AFB1 administration.

DISCUSSION

Results form bioactivity studies indicate that the leaves of *V. amygdalina* del may have higher bioactivity in low doses than *V. colorata.* It also indicates that the bioactivity and pharmacological properties of these leaves may reside more in the methanolic fraction.

Alkaline phosphatase is a cholestasis marker being a membrane bound enzyme. Elevation in its activity is associated with hepatobiliary diseases and is more marked the more complete the obstruction (Teitz, 1987). The variance in the pattern of elevation of alkaline

Table 3. Effects of feeding of two varieties of *Vernonia* on some serum enzymes.

S/N	Group treatment	ALT (I.U/L)	AST (I.U/L)	ALK (I.U/L)
1.	Basal diet	12.00 ± 0.00^{a}	12.67 ± 3.00^{a}	34.12 ± 3.75^{a}
2.	Basal diet + AFB_1	12.00 ± 0.00^{a}	6.67 ± 3.06^{a}	160 ± 17.85^{a}
3.	*Vernonia colorata* diet	36.00 ± 0.00^{c}	26.66 ± 11.55^{a}	27.22 ± 4.10^{a}
4.	*Vernonia amydalina* del diet	8.0 ± 3.46^{a}	14.00 ± 6.93^{a}	140.15 ± 1.78^{b}
5.	*Vernonia colorata* diet + AFB_1	37.67 ± 0.29^{c}	28.00 ± 19.29^{a}	421 ± 195.91^{c}
6.	*Vernonia amydalina* del diet = AFB_1	5.00 ± 1.00^{a}	30.00 ± 16.00^{a}	168 ± 76.32^{a}

*Means with the same superscripts are not significantly different.

Plate 3. Liver section showing hyperplasia of the duct epithelium. [H and E stain X 180] in rats fed *Vernonia colorata*.

phospatase activity in this study with the feeding of the indicative of differences in patterns of toxic lesion the liver associated with the two different cultivars. This inference is further substantiated by the histopathological studies which indicate milder lesions in periportal area of the liver in the animals fed *V. amygdalina* del with AFB_1 treatment. Severe hepatocellular lesions with parenchymal cell necrosis is usually accompanied by a marked elevation of the mitochondrial enzyme AST which is distinctly increased over ALT. This pattern is seen in the group fed *V. colorata* with or without AFB_1 (Plates 3 and 4). These findings are in consonance with the findings of Arhoghro et al. (2009) who also reported hepatoprotective effects of *V. amygdalina* against Carbon tetrachloride induced hepatic damage. Ijeh and Obidoa (2004) reported the induction of Phase 11 biotransformation enzymes such as glutathions S- tranferase activity in rats fed *V. amygdalina* incorporated diets along with AFB1. GST is known to biotransform AFB_1 to less toxic metabolites thereby conferring heepatoprotection.

ALT and AST activities in rats fed the *V. amygdalina* without AFB_1 did not differ significantly from rats fed basal diet indicating that the vegetable diet had no chronic toxicity effects. The elevation over ALT is marked in group 5 compared to group 6 indicating a possibility that this vegetable potentiates the toxic effects of AFB_1. There is a variance in the pattern of increase in AST activities in the groups fed *V. colorata* only and *V. amygdalina* del only without AFB_1 treatment. AST is a cellular enzyme of the liver lobules and changes in its activity vary in serum depending on the severity, type and stage of liver damage (Rosalki and Wilkrion, 1976).

ALT and AST activities in group 6 fed the *V. amygdalina* del compares with those fed the basal diet indicating that the feeding of this vegetable variety may not be toxic to the liver cell itself but could potentiate the toxic effects of AFB_1 possibly by inducing phase one microsomal enzymes. This has been observed by earlier

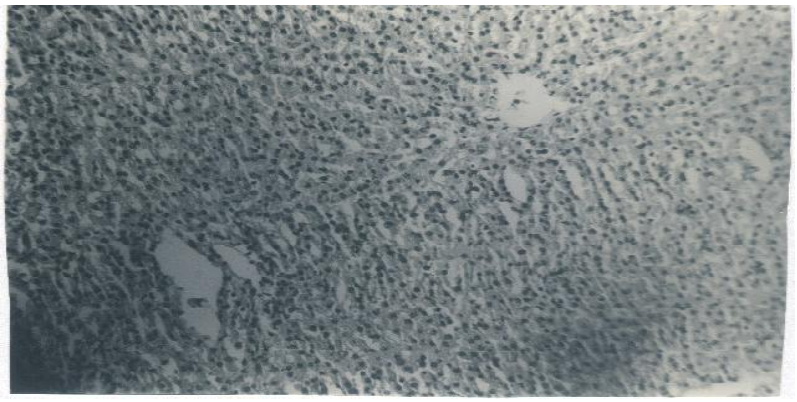

Plate 4. Photomicrograph of liver sections showing proliferation of bile ductules [H and E stain X 180] in rats fed *Vernonia colorata* diets +AFB_1.

workers (Ezekwe, 1991).

Conclusion

Our studies are indicative that the two varieties of *Vernonia* under study may have different pharmacological properties and effects on body organs especially the heart and liver. Our findings are indicative that while *V. amygdalina* del may be hepatoprotective against certain forms of liver damage it may cause obstructive liver damage as indicated by elevation of alkaline phosphates activity. *V. colorata* may potentiate the toxic effects of toxins such AFB_1 as indicated by elevation of alanine amino transminase activity and aspartate amino transaminase activity.

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