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

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Accepted 21 September, 2010

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 AFB₁. 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 B₁ over a 12 week period. Bioactivity studies using brine shrimp lethality test indicate that methanolic extracts of V. amygdalina and V. colorata had higher bioactivity (ED₅₀ 121.47 µg/mL and 761.8 µg/mL respectively) than their aqueous extracts (819.5 and 1,999.85 µg/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 AFB₁ treatment but showed no significant increase in groups fed V. amygdalina with or without AFB₁. Treatment of V. colorata fed animals with AFB₁ (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 AFB₁. Administration of AFB₁ 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 + AFB₁ 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 AFB₁. 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 amygdalina, Vernonia colorata, AFB₁, 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) antiparasitic activity (Ohigashi, 1995) and antimicrobial activity against organisms commonly incriminated in wounds (Ijeh et al., 1996).
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).

**Enzyme assays**

Assays of Alanine amino transaminase 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 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.

**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 AFB1 was administered *per os* by intubation for ten days to groups, 2, 4, and 6 commencing on the 10th day of feeding the animals (post-equilibration). After AFB1 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.

**Terpenoid extracts from** *V. amygdalina* **have been demonstrated to have hepatoprotective effects against Carbon tetrachloride induced liver damage in rats. Arhogho 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 than in developed countries and temperate regions of the world (Liu and Wu, 2010). Numerous epidemiological studies have observed a correlation between areas of high aflatoxin B1 exposure and high incidence of hepatocellular carcinoma (Jackson and Groopman, 1999).

The feeding of AFB1 along side *V. amygdalina* in this study is borne out of the need to investigate its possible effects on AFB1 toxicity since research evidence has shown that certain dietary constituents can potentiate or reduce the potency of toxins such as AFB1. AFB1 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).

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

<table>
<thead>
<tr>
<th>Ingredient/feedstuff</th>
<th>Diet 1</th>
<th>Diet 11</th>
<th>Diet 111</th>
</tr>
</thead>
<tbody>
<tr>
<td>Maize (flour)</td>
<td>50.50</td>
<td>50.50</td>
<td>50.50</td>
</tr>
<tr>
<td>Crayfish</td>
<td>4.29</td>
<td>3.36</td>
<td>3.36</td>
</tr>
<tr>
<td>Groundnut (meal)</td>
<td>9.39</td>
<td>7.43</td>
<td>7.43</td>
</tr>
<tr>
<td>V. colorata</td>
<td>-</td>
<td>4.61</td>
<td>-</td>
</tr>
<tr>
<td>V. amygdalina. del</td>
<td>-</td>
<td>-</td>
<td>4.33</td>
</tr>
<tr>
<td>Bone meal</td>
<td>4.00</td>
<td>4.00</td>
<td>4.00</td>
</tr>
<tr>
<td>Pre-mix</td>
<td>0.50</td>
<td>0.50</td>
<td>0.50</td>
</tr>
<tr>
<td>Oil</td>
<td>5.00</td>
<td>5.00</td>
<td>5.00</td>
</tr>
<tr>
<td>Corn-flour</td>
<td>26.50</td>
<td>24.50</td>
<td>24.88</td>
</tr>
<tr>
<td>Total (approx)</td>
<td>100.00</td>
<td>100.00</td>
<td>100.00</td>
</tr>
</tbody>
</table>

**Table 2.** Brine shrimp Lethality tests.

<table>
<thead>
<tr>
<th>Extract</th>
<th>ED (50 μglm/l)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Aqueous extract V.a var</td>
<td>1,999.85</td>
</tr>
<tr>
<td>Methanolic extract V.a var</td>
<td>761.89</td>
</tr>
<tr>
<td>Aqueous extract V.a del</td>
<td>819.59</td>
</tr>
<tr>
<td>Methanolic extract V.a del</td>
<td>121.47</td>
</tr>
</tbody>
</table>
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 + AFB1.

V. amygdalina del showed the highest bioactivity (ED$_{50}$ 121.47 μg/ml) while aqueous extracts of Vernonia colorata showed the lowest bioactivity (ED$_{50}$ 1,999.8 μg/ml). Remarkable changes in liver pathology phosphatase activity (Table 3) increased significantly in all groups treated with AFB1 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.

<table>
<thead>
<tr>
<th>S/N</th>
<th>Group treatment</th>
<th>ALT (I.U/L)</th>
<th>AST (I.U/L)</th>
<th>ALK (I.U/L)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1.</td>
<td>Basal diet</td>
<td>12.00 ± 0.00^a</td>
<td>12.67± 3.00</td>
<td>34.12 ± 3.75</td>
</tr>
<tr>
<td>2.</td>
<td>Basal diet + AFB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>12.00 ± 0.00^a</td>
<td>6.67 ± 3.06</td>
<td>160 ± 17.85</td>
</tr>
<tr>
<td>3.</td>
<td><em>Vernonia colorata</em> diet</td>
<td>36.00 ± 0.00^c</td>
<td>26.66 ± 11.55</td>
<td>27.22 ± 4.10</td>
</tr>
<tr>
<td>4.</td>
<td><em>Vernonia amygdalina</em> del diet</td>
<td>8.0 ± 3.46^a</td>
<td>14.00 ± 6.93</td>
<td>140.15 ± 1.78</td>
</tr>
<tr>
<td>5.</td>
<td><em>Vernonia colorata</em> diet + AFB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>37.67± 0.29^c</td>
<td>28.00 ± 19.29</td>
<td>421 ± 195.91</td>
</tr>
<tr>
<td>6.</td>
<td><em>Vernonia amygdalina</em> del diet = AFB&lt;sub&gt;1&lt;/sub&gt;</td>
<td>5.00 ± 1.00^a</td>
<td>30.00 ± 16.00</td>
<td>168 ± 76.32</td>
</tr>
</tbody>
</table>

*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*.

Phosphatase 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<sub>1</sub> 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<sub>1</sub> treatment. These findings are in consonance with the findings of Arhogho 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-traferase activity in rats fed *V. amygdalina* incorporated diets along with AFB<sub>1</sub>. GST is known to biotransform AFB<sub>1</sub> to less toxic metabolites thereby conferring hepatoprotection.

ALT and AST activities in rats fed the *V. amygdalina* without AFB<sub>1</sub> 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<sub>1</sub>. 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<sub>1</sub> 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<sub>1</sub> possibly by inducing phase one microsomal enzymes. This has been observed by earlier
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Plate 4. Photomicrograph of liver sections showing proliferation of bile ductules [H and E stain X 180] in rats fed Vernonia colorata diets + AFB1.

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 AFB1, as indicated by elevation of alanine amino transaminase activity and aspartate amino transaminase activity.

ACKNOWLEDGEMENTS

The work was carried out in Prof. O. Obioda’s laboratory in the Department of Biochemistry, University of Nigeria, Nsukka. We acknowledge financial assistance given to Dr Ifeoma Irene Ijeh by the Abia State University Uturu, Nigeria.

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